VOLUME 6 AUGUST 1957 NUMBER 3

AUSTRALASIAN ANNALS OF MEDICINE

Journal of The Royal Australasian College of Physicians

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The journal is published quarterly in February, May, August, and November. The subscription rate is four pounds per annum (one pound per issue), payable in advance to The Royal Australasian College of Physicians, 145 Macquarie Street, Sydney. Advertising rates may be obtained on application to Australasian Medical Publishing Company Limited, Seamer and Arundel Streets, Glebe, Sydney.

AUSTRALASIAN ANNALS OF MEDICINE

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THE CHALLENGE OF NORTH QUEENSLAND FEVERS[1]

E. H. DERRICK[2]

From the Queensland Institute of Medical Research, Brisbane

SUMMARY

The distribution of fevers in North Queensland may largely be correlated with latitude, altitude and rainfall.

In the Gulf country and on mining fields in the early days, typhoid and malaria were major causes of fever. Other cases were commonly designated " typho-malaria ".

On the Pacific coast, the incidence of fevers has been high since the eighties. At first obscure in nature, they have been shown to consist mainly of leptospirosis and scrub typhus.

Many minds contributed to the elucidation of the northern fevers ; the staff of the Australian Institute of Tropical Medicine at Townsville played a notable part from 1910 to 1930. Repeatedly, an advance had to await the coming of the right man.

The disappearance of typhoid and malaria from the mainland of North Queensland is a public health victory. The means to prevent dengue and to lessen scrub typhus are to hand, but the prevention of leptospirosis is an unsolved problem.

Splendour of mountain and plain,
Pageant of forest and stream,
Bounteous sunshine and rain,
Beauty surpassing man's dream,

Terror of cyclone and flood,
Torment of sting and of bite,
Peril that lurks in the mud,
Reptile, mosquito and mite,

Courage that conquers the wild,
Urging the pioneer forth,
Strivings to banish disease—

These shape thy destiny, North !

THE traveller by air along the North Queensland coast looks down on an enchanted land.

These headlands below him, these bays and islands, were named by Captain Cook and the intrepid navigators that followed. These rivers and mountains commemorate explorers and pioneers and early notables. In that maze of swamp and watercourse, Edmund Kennedy's expedition was entangled at its very start. In those dense jungles, Christie Palmerston, the outlawed son of a Prime Minister, established his domain among the aborigines. These formidable ramparts for years forbade transport between coast and tableland, until they were pierced by the skill of surveyor and engineer. Murdering Point, Wreck Bay and Battle Camp tell of the cost of occupation. In contrast, here is peaceful Dunk Island, where the " Beachcomber " found a tranquillity the city had not given him.

But it is not the beauty of the North Queensland coast or the romance of its history that inspires our theme, but the particular hazards to health that it presents. Like that other Eden, this enchanted land has its perils. They lurk in its waters in forms ranging from crocodile to leptospira and, as various as stinging tree and trombicula, infest its jungles. Prodigality of warmth and rainfall not only give abundant growth of sugarcane and tropical fruit, but also multiply agents and vectors of disease.

Bird, mammal and reptile are liable to have parasites on the skin, in the blood and in the intestine. Even flies and mosquitoes have their parasites ; a certain mite attaches itself to a mosquito larva in the water, maintains

[1] Based on an address delivered to the Annual Meeting of the National Association for the Prevention of Tuberculosis in Australia at Brisbane, September 21, 1956.

[2] Deputy Director, Queensland Institute of Medical Research.

its attachment during the metamorphosis, and then accompanies the adult mosquito into the air.

SELECTIVE CLIMATOLOGY

It needs to be made clear that there is not just one climate in North Queensland, but many. At least four types are illustrated in Figure I, a profile at about 17·4° south latitude.

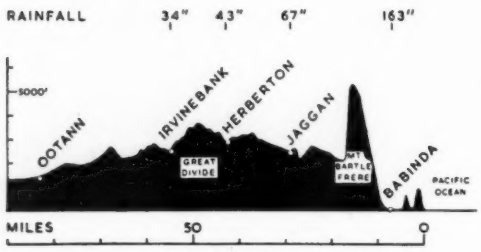

FIGURE I

Profile of the Tableland region of North Queensland, slightly obliquely, at about 17·4° latitude

A feature that strongly influences the local climate is the mountain mass that culminates in Bartle Frere (5275 feet). This provides a cooler region—the mean temperature at Herberton (67·55° F.) is nine degrees less than

FIGURE II

Irvinebank in 1926. The flues on the right belong to the disused tin smelter

that of Cairns, and even less than that of Brisbane (68·9° F.)—as well as a heavy rainfall, particularly on its seaward side.

One type of climate is found on the very wet coastal plain, where sugarcane flourishes and dense jungles abound; this is the realm of tropical diseases. Next come the tablelands at an elevation of 2000 to 3000 feet. Dwellers on the steamy coast are fortunate to have close by these invigorating tablelands for recreation and convalescence. Their eastern part has a rainfall less than the coast, but still ample for dairying and agriculture and for rain forest, and sufficient to encourage the spread of certain infective agents. The western part of the tablelands is comparatively dry and is largely mining country. It is exemplified by Irvinebank (Figure II) and Mount Garnet. Miners' phthisis is not uncommon, but tropical diseases are rarely met with. Still further west, the land gradually falls away to the hot, dry plains that border the Gulf.

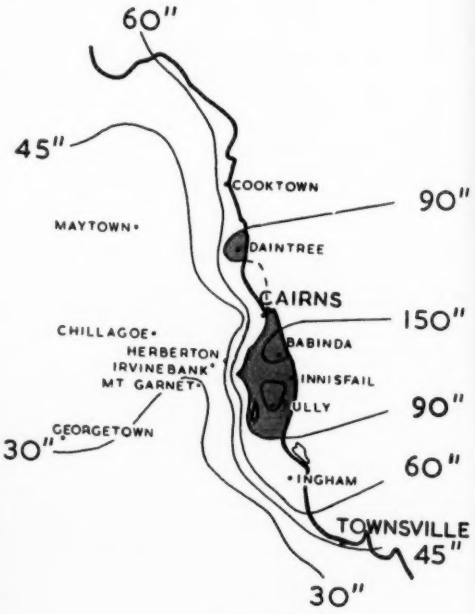

FIGURE III

Rainfall map of part of North Queensland, including the wettest area in Australia

The rainfall map is most instructive (Figure III).

The places in North Queensland where leptospirosis has been a major problem in the last few years have an average annual rainfall of 90 inches or more. The leptospira usually spreads from animal to man by water or wet ground, and heavy rain encourages the spread. It is true that leptospirosis occurs widely with a lesser rainfall; Ingham, where it averages 81 inches, had a severe outbreak in 1933–1934, and even Winton, on 16 inches, can boast of one

recorded case. But leptospirosis is not a problem in the canefields around Mackay, with 60 to 70 inches.

The mite that transmits scrub typhus, *Trombicula deliensis*, is very susceptible to drying, and scrub typhus is not found where the rainfall is below about 60 inches. Tick typhus is less exacting, and will manage down to about 45 inches. The spread of hookworm is favoured by wetness and it, too, is rarely found where the rainfall is less than 45 inches.

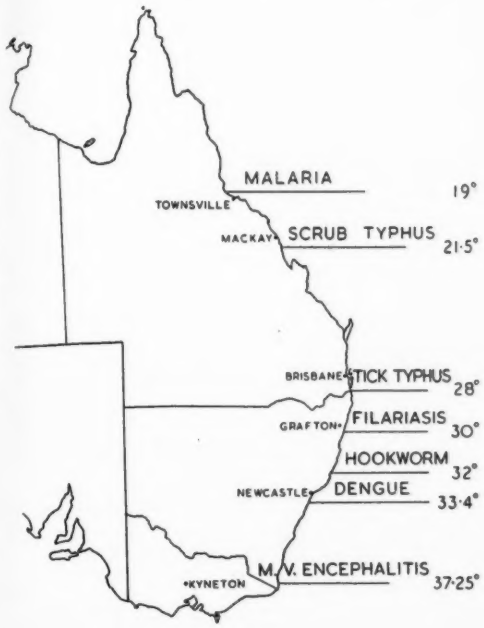

FIGURE IV
Limiting latitudes in Australia for certain "tropical" diseases

Latitude is also important. Along the Pacific coast of Queensland, the mean temperature rises about 1° F. for each 1·4° decrease in latitude.

There is no appreciable risk of malaria in Queensland south of the nineteenth parallel. That statement needs a little discussion. Last century, widespread outbreaks of malaria were reported south of this line, but that was before the days of microscopical verification, and the diagnosis in many is open to question. It is true, however, that authenticated cases of malaria have occasionally arisen much further south. During the last war, a soldier recently returned from New Guinea and a schoolboy relative spent a holiday together at Canungra in the south of Queensland at latitude 28° S. The boy developed malaria, evidently transmitted from the soldier by a local anopheline mosquito. Similar cases are listed by Ford (1950), even as far south as Melbourne, 38° S., but they are exceptional. The efficient vector of malaria in Australia, *Anopheles farauti*, has not certainly been found south of Townsville, 19·3° S.

The southern limit of scrub typhus is at 21·5° S. Tick typhus has been reported at 28° S., but probably exists further south. Filariasis ceases at 30° S., and hookworm at 32° S. The 1926 epidemic of dengue swept down to Gosford, 33·4° S., and that may be regarded as the southern limit of effective populations of *Aëdes ægypti*. Murray Valley encephalitis, with the advantage of avian transport, reached in 1951 to Kyneton, 37·25° S.

These geographical boundaries of disease (Figure IV) are not unalterably fixed. As well as fluctuations with season, there could be big changes in either direction. The accidental introduction of some particular species of *Anopheles* might make malaria endemic even in New South Wales. In Africa and South America, it exists as far south as 29·5° and 30·3° S. respectively. There is no climatic reason why scrub typhus should halt at 21·5° S.; in Japan it occurs at a latitude of 39° N.

On the other hand, intelligently directed control measures can sweep the tide back. Since the war, malaria has disappeared from the mainland of Queensland. And the last epidemic of dengue in North Queensland did not reach Brisbane, because Brisbane is now free of *A. ægypti*.

By a judicious blend of altitude, latitude and rainfall, man or microorganism may select a climate to his liking.

"LET KNOWLEDGE GROW FROM MORE TO MORE"

When one wayfarer went north in 1926, he was interested, and at times bewildered, to meet diseases uncommon or absent in the south. He saw his first case of yaws in an aboriginal child, and his first of *granuloma venereum*. He was called to treat muscular cramps in canecutter and miner due to loss of salt in the sweat, and to adjudicate on suspect lepers. He met fevers of obscure nature. He was told that sprue was endemic in Babinda and was, therefore, careful not to drink any water while his train halted there. He was shocked to observe that hookworm infestation could kill a strong man.

In August, 1927, a man aged thirty-nine years, who had been working in the hills behind Cardwell, was admitted to the Innisfail Hospital. He complained of diarrhœa, pains in the lower part of the abdomen and weakness, and was obviously very anæmic. He was regarded as probably suffering from sprue and was treated accordingly, but he gradually sank and died. At autopsy, hundreds of hookworms were found in the duodenum, starting six inches from the pylorus, and in the jejunum. It was a jolting and humbling experience, for hookworm had not been considered in the diagnosis, even in consultation. As an anticlimax, an officer of the Hookworm Campaign made one of his periodic visits to Innisfail shortly afterwards. Actually, James Hogg, of Goodna, had reported a fatal case in 1889.

The wayfarer was not the first to find his medical training inadequate for tropical Australia. As far back as 1901, Frederick Goldsmith, of Darwin, pointed out to the Intercolonial Medical Congress, then meeting in Hobart, that many diseases met with in tropical Australia were untouched in the ordinary medical course. Inspired by him, the Congress resolved that: "It is desirable that a School of Tropical Medicine and Research should be established for the scientific and systematic investigation of tropical diseases in Australia." Brisbane was suggested as the most suitable site to carry out investigations.

The seed thus planted at Hobart was nurtured by the Universities, particularly that of Sydney, stimulated by grants from the Commonwealth and Queensland Governments, especially the former, and blossomed in 1910 as the Australian Institute of Tropical Medicine (Figure V). That this was established in the tropics, at Townsville, was due to the advocacy of George Frodsham, D.D., Bishop of North Queensland.

The founding of the Tropical Institute was an outstanding event. It was the first Medical Research Institute in Australia, antedating by six years the Walter and Eliza Hall Institute in Melbourne. It was also an outstanding event in the medical history of North Queensland. For twenty years it was a stronghold and an outpost of scientific medicine in the north. Anton Breinl, who had established a reputation for his researches into tropical fevers in Africa and South America, became the first Director, and he was supported by an able team: Henry Priestley and William Nicoll, Research Assistants; William Young, Biochemist; Frank Taylor, Entomologist; and Jack Fielding, Laboratory Assistant. The spotlight fell on a wide variety of tropical diseases, and also on the particular physiology of North Queenslanders. In 1922, the original research team having left for other fields, the work of the Institute was reorganized with Raphael Cilento as Director. Alec Baldwin and George Heydon became associated with it, and Taylor returned. Administrative control passed to the Division of Tropical Hygiene of the Commonwealth Department of Health. The scope of the work broadened, and an original aim of the Institute was implemented—the provision of a course of study leading to the Diploma of Tropical Medicine.

The full story of the Tropical Institute, with its triumphs, vicissitudes and untimely end, and the personalities that founded and staffed it (Figure VI), should one day be told. Its investigations are recorded in over 150 published reports, to which the inquirer turns again and again for information on the incidence and distribution of tropical diseases in the north.

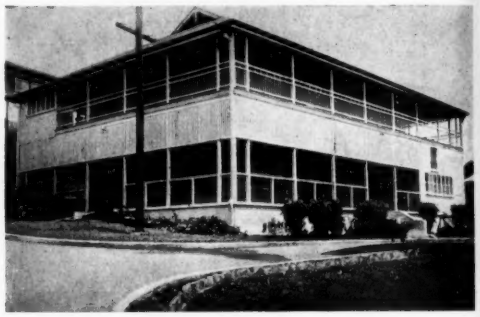

FIGURE V

The Australian Institute of Tropical Medicine, Townsville, which functioned from 1910 to 1930

In 1918, reinforcements to tropical research arrived in North Queensland in the form of the Hookworm Campaign, instituted by Commonwealth and State Governments in conjunction with the International Health Board of the Rockefeller Foundation.

Another significant development was the establishment of three Commonwealth Health Laboratories in tropical Queensland. The first was opened in 1922 in Townsville in conjunction with the Tropical Institute; one at Rockhampton followed in 1924 and one at Cairns (Figure VII) in 1928. Their purpose was to provide a laboratory service for clinical and preventive medicine. This routine service was essential in consolidating the ground won by research; in addition, the opportunity was grasped from time to time to carry out further valuable investigations.

In 1930, the clock was turned back. The research and teaching functions of the Tropical

Institute were transferred from Townsville to Sydney, and the chapter that had opened so auspiciously in 1910 was closed. There were reasons for the transfer, at least as far as teaching was concerned—the number of students who had taken the diploma course was disappointing. But it left a gap in the investigation of North Queensland problems that was only partly filled by the Health Laboratories and occasional research expeditions from the south.

FIGURE VI

Personalities associated with the Australian Institute of Tropical Medicine: (A) G. H. Frodsham, D.D., Bishop of North Queensland, 1902–1913, co-founder. (B) A. Breinl, Director, 1910–1919. (C) R. W. Cilento, Director, 1922–1928. (D) A. H. Baldwin, Acting Director, 1924–1930

An advance on the educational front came in 1936. Tropical medicine was included as an integral part of the degree course when a Faculty of Medicine was inaugurated at the University of Queensland. On the research front, the latest reinforcement has been the Field Station of the Queensland Institute of Medical Research, founded at Innisfail in 1951. Rebuilt and expanded Commonwealth Health Laboratories at Cairns and Townsville were opened in 1954 and 1955.

Thus, over the years, there have been some notable strivings towards the twin ideals of research and education put forward in 1902, although their fulfilment is by no means complete.

THE PRICE OF PIONEERING

The newcomer to the North hears many accounts of "fever"—accounts woven of mystery and tragedy and coloured by the imagination of the narrator. One story, for instance, is that, when the Mourilyan Harbour tramway was constructed in 1883, a man died for every sleeper laid. As there would be about 18,000 sleepers on that line, and the total population of the district then under one thousand, it is clear that the teller of tales in North Queensland can hold his own with his fellows anywhere.

But there was no need for hyperbole. The facts were striking enough.

Official registrations record that, in the first fifty years of Townsville's existence, over 500 of its citizens died of typhoid (Mitchell, 1925). Typhoid here includes also "fever", "gastric fever", "colonial fever", "continued fever" and "low fever".

The early sixties saw a steady northward advance of pastoral occupation, an advance accompanied by the opening of ports—Bowen in 1861, Townsville in 1863 and Cardwell in 1864. From this period, "fever and ague" find repeated mention. E. B. Kennedy, who roamed widely over North Queensland, stated (1870) that it was rare to escape "fever and ague" in new country. Sidney Hunt (1890) recorded that "fever and ague" was a comparatively common complaint in Hughenden in 1885, but the "slow fever" had gradually taken its place. Both of these were apparently regarded by him as varieties of malaria. He quoted the widespread belief amongst dwellers in the bush that as a district became stocked up, the fever died out. In 1893, he reported that 221 of 1000 admissions to the Hughenden Hospital had been for fever.

By 1864, the pastoral advance had reached the Gulf of Carpentaria and, in May, 1865, Burketown was founded to be the port for the "Plains of Promise", as Captain Stokes, in a lyrical outburst, had named this area in 1841. From April to June, 1866, Burketown was devastated by an epidemic. At least 50 of the 76 inhabitants died, and the remainder were reduced to miserable skeletons (Uhr, 1868; Palmer, 1903; Elkington, 1912). Others died on surrounding stations. In that way, for

many, was the promise fulfilled. Death from fever claimed also two noted explorers who called in at Burketown in that year—in June, Duncan MacIntyre, who was leading an expedition to search for traces of Leichhardt, and in November, Frederick Walker, who had travelled across from Townsville to select a route for a telegraph line. Aitken White considered the epidemic to be the *febris typhoides*, as the fever was continued and contagious and accompanied by the daily recurrence of the *taches rosées lenticulaires* of Louis. William Landsborough, who had explored the Burketown area in 1861 looking for Burke and Wills, returned in 1866 as Police Magistrate and, because of the fever, moved his headquarters to Sweers Island. For some years, until 1875, the original site of Burketown was completely deserted.

That disastrous visitation to the first settlement on the Gulf so early in its history firmly established the tradition of "Gulf fever". This term was widely and loosely used for the next thirty years or more. No epidemic of such severity ever struck Burketown again. There is a story ("Local Topics", 1868) that two doctors arrived there to make their fortunes by treating the prevailing endemic fever, but failing in their aim one obtained employment as a publican and one as an overseer. However, fever cases were a continuing reality in the Gulf. In 1889, Thomas Dyson recorded that, for the previous three years, they had constituted one-fifth of all cases in the Burke District Hospital at Normanton.

The following account of an epidemic at Mackay witnessed by J. T. S. Bird is recorded in the *Mackay Mercury* of September 5, 1923.

> Beyond isolated cases of fever, Mackay was regarded as particularly healthy. For all that it was in a terrible insanitary state, and practically there was no drainage whatever. In January, 1870, there were very heavy rains and the town and whole country for miles was thoroughly soaked. The Pioneer arose to within a foot of overflowing its banks, and every hollow was filled with water. In the intervals of the showery weather the hot sun produced a sweltering heat, and the stench in places was far from pleasant. In a few weeks one of the pilots named Stephenson died somewhat suddenly from fever, and a week or two after, cases of very serious nature were reported all over the town. Dr. McBurney declared it to be typhoid of a somewhat malignant type. The sergeant of police, a man named Harry Smith, Dalgleish, the butcher, and others died after a few days' illness. At the same time there was another form of low fever prevalent, and when the doctor himself got sick, quite a scare occurred. Happily Dr. McBurney got better after a few days, and Ben Clark, Mrs. White, and a few other very bad cases began to slowly mend. There were many deaths from the disease, considering the small population, but a change in the weather had a good effect, and by the end of March the danger was practically over. This untimely epidemic did some service by impressing on the municipal authorities the urgency of forming the streets and draining the foul pools of stagnant water.

The discovery of gold in North Queensland also brought fever in its train. The first rush was to the Cape River in 1867. It was followed by numerous important finds, including Charters Towers and the Palmer in 1872. Mining camps were swept by epidemics, and the mortality at times was high.

W. R. O. Hill (1907), who was Gold Warden at the Palmer from 1876 to 1878, thus records his experience: "We were often on duty away among the ranges, two or three weeks at a stretch . . . We were rarely free from fever, and I had sometimes to lie down in the dust on the main road, shivering like an aspen leaf for an hour or two, and after this came a raging fever which generally made me quite delirious." Because of the continual attacks of fever, as well as the severity of his work, Hill hailed with delight his transfer away from the Palmer.

There are some good clinical descriptions of those early fevers by northern physicians—among others, White in 1867, Philip James of Croydon in 1891, and, to go outside Queensland, Goldsmith of Darwin in 1901. It is the achievement of these physicians that, by clinical observation, they lifted out of the confusion two definite fever entities—malaria and typhoid. To over-simplify the picture: in some cases the fever was remittent or intermittent, the temperature rising and falling steeply each day or on alternate days, and it responded to quinine (see Goldsmith's charts, "D.C." and "O.K."); such a course was more or less characteristic of malaria. In other cases the fever was continued (that is, with little daily fluctuation) and prolonged, and did not respond to quinine; that was characteristic of typhoid. So far, there was a measure of agreement. But very many cases did not conform to those types, and about them the utmost confusion prevailed.

Figure VIII is the chart of Goldsmith's patient "H.W." The fever was prolonged, as in typhoid, but it was remittent, as in malaria. It was typical of neither disease, but combined features of both. Goldsmith's diagnosis was—not unreasonably—typho-malaria, his emphasis being on malaria. What that case actually was is a matter of speculation; there would be a number of possibilities. Goldsmith considered that the remissions were not due to the five grains of antipyrin he administered whenever the temperature reached 102° F., for they occurred also without antipyrin.

The term " typho-malaria " had wide sanction in those days. James notes that it was current in India. Cushing (1940) relates that Osler, at his clinic in Baltimore, organized in 1889, insisted that " no diagnosis of malaria be made

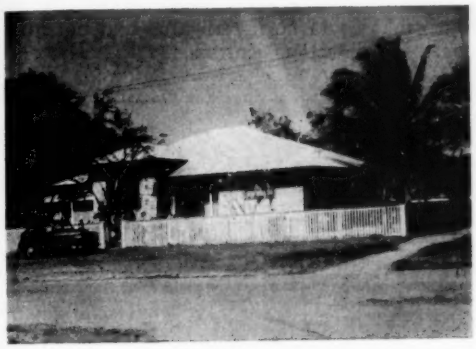

FIGURE VII

Commonwealth Health Laboratory, Cairns, 1928–1954. In this unpretentious building, R. Y. Mathew and W. G. Heaslip carried out their investigations on scrub typhus

without a microscopic demonstration of the parasite. It was a most important matter for nearly every fever in the South at the time was loosely called typho-malaria and treated with quinine."

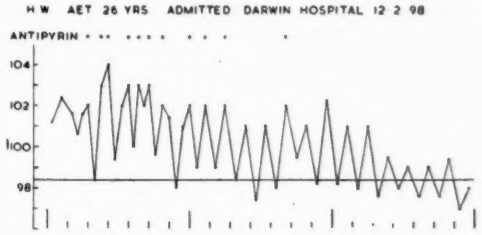

FIGURE VIII

Temperature chart in a case of " typho-malaria ", reproduced from Goldsmith (1901)

In North Queensland, also, the term was often used quite loosely. One old-time nurse, discussing the fevers of the early days, related that some doctors thought they were typhoid, and some thought they were malaria, but one clever doctor found out they were really typho-malaria.

James thus disposed of " Gulf fever ". The term, he said, " is used vulgarly to describe any disease it which the patient becomes hot. It may mean remittent fever, or typhoid, or simple continued fever or simple pyrexia from solar effects, or other disease not of a specific nature, but which may be accompanied by a rise of temperature."

The term " colonial fever ", widely current in Brisbane and southern States, was little used in North Queensland. By the time settlement began in the north, " colonial fever " had become generally accepted as synonymous with typhoid.

The recent isolation by Pope and Carley (1956) of a species of *Borrelia* from rats at Richmond suggests that relapsing fever may have contributed to the fever cases in north-western Queensland.

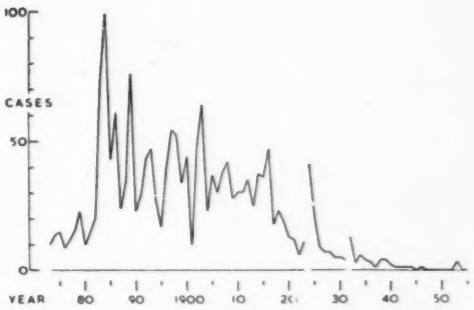

FIGURE IX

Number of deaths from typhoid annually, showing its virtual disappearance. From 1873 to 1923, the figures are for the four Registration Districts comprising North Queensland and are taken from Cumpston and McCallum (1927). After 1923, figures for North Queensland alone not being available, larger areas which include North Queensland have been used—from 1924 to 1931, Queensland excluding Brisbane; from 1932 to 1955, tropical Queensland. The figures for 1932 onward include also paratyphoid. Figures from 1924 to 1955 by courtesy of the Queensland Government Statistician

" RING OUT OLD SHAPES OF FOUL DISEASE "

Typhoid was a severe infection—about one in seven patients died—and the certified deaths give a good index of its incidence over the years (Figure IX). It became a menace on the goldfields and railway construction camps in the north. The sudden crowding in of men with no ordered sanitation produced just the conditions for explosive outbreaks. The high mortality in the eighties came mainly from Charters Towers, but there was a good deal also from Townsville and Mackay. Graham Browne (1890) reported 137 deaths from typhoid

in Charters Towers in the seven years from 1883 to 1889, and described the deplorable sanitation there in 1882—rows of cesspits on the flats in close proximity to the wells from which drinking water was drawn in dry weather. In wet weather the contents of the cesspits were flooded out over the surface of the soil. In Croydon, also, James observed " the scandalous neglect of ordinary sanitary precautions which is the rule in North Queensland ".

Browne's account of typhoid at Charters Towers may be supplemented by a patient's point of view. J. G. Eastwood reported that, in May and June, 1887, an epidemic of " malarial fever and ague " swept up and down the Great Northern railway line, then being extended, filling the hospitals at Townsville, Charters Towers and Hughenden with fever patients. He was at the time a lengthsman on the line and spent three weeks with the fever in Charters Towers Hospital. He had also had a " heavy touch of malarial fever " at Hughenden in the previous year (Collinson, 1946).

The decline and fall of typhoid is instructive.

With the passing of the frontier phase of goldfield life, when health standards were of minor concern, and with the introduction of sanitary services and piped water supplies, the peak mortality of the eighties subsided.

The next twenty-five years were up and down. On the whole, there was a slight improvement in the death rate. This is not brought out in Figure IX, which takes no account of the steadily increasing population.

During this time important influences were at work which were to bear fruit later. Firstly, following the discovery of the typhoid bacillus by Eberth in Germany in 1880, there was developing a clearer understanding of the disease and how it was spread. The former vague ideas of association with cesspit or polluted water gave place to knowledge of infection with a specific bacillus which must have come, directly or indirectly, from a patient or carrier. Flies could transmit infection. Gradually the newer knowledge spread and became general. But Cumpston and McCallum (1927) tell us that " it was only with the commencement of the twentieth century that Australia as a whole developed an informed and sensitive sanitary conscience ". John Elkington, the Commissioner of Public Health in Queensland from 1910 to 1913, strove forcefully to hasten the development of that conscience; his annual reports still make stimulating reading.

Secondly, laboratory aids were devised. The cultural test for the typhoid bacillus and the agglutination test, introduced in 1896, increased the accuracy of diagnosis, allowed the infectivity of a convalescent to be determined, indicated when he could safely be released from isolation, and made possible the detection of carriers. Outbreaks could be investigated and potential sources of infection controlled.

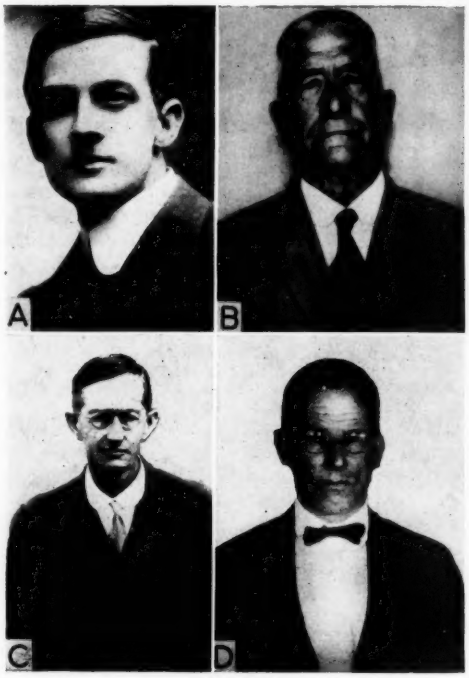

FIGURE X

Investigators of malaria: (A) R. A. O'Brien, who practised in Cairns, 1904–1908, and was the first to demonstrate malaria parasites microscopically in North Queensland. (B) J. S. C. Elkington, Commissioner for Public Health, Queensland, 1910–1913, who was impressed by the heavy mortality ascribed to malaria in the early days of the Gulf country. (C) F. H. Taylor, Entomologist to the Australian Institute of Tropical Medicine, 1911–1930, who made many surveys of anopheline and other mosquitoes. (D) G. A. M. Heydon, who surveyed Cairns for malaria in 1927, and, in the 1942 outbreak there, proved the vector was *Anopheles farauti*

Agglutination tests for typhoid in North Queensland were carried out in Brisbane by Jefferis Turner in 1897, using blood dried on notepaper and posted from Charters Towers. He reported typical reactions. Specific tests became more readily available in the north when

the Tropical Institute opened in 1910. Breinl and Priestley soon formed two conclusions: some of the cases clinically diagnosed as typhoid were due to some other cause; the clinical manifestations of typhoid in the tropics differed in many respects from those of typhoid in the south.

Thirdly, the prophylactic value of an anti-typhoid vaccine had been established in England by Wright and Leishman. In 1913, J. Booth-Clarkson used vaccine to combat an outbreak at Winton, where there were 77 cases with 12 deaths. The vaccine was prepared by John Harris, Director of the Laboratory of Microbiology and Pathology, Brisbane. Vaccination was strongly advocated wherever typhoid appeared in Queensland.

All these influences, and perhaps others, culminated in a striking fall in mortality that began in 1917 and continued during the twenties and thirties. The improvement was punctuated by outbreaks here and there. A series of 25 cases in Cairns in 1928 was puzzling, for although the cases were undoubtedly typhoid, the agglutination test with a stock strain of the typhoid bacillus gave unsatisfactory results. One possibility was that the series was due to an aberrant strain of the typhoid bacillus; a more likely one that the stock strain had become unsuitable for use in the test.

The Cloncurry mining field in the dry north-west of Queensland has figured prominently in the typhoid story. During an outbreak in 1913, the medical officer of health, Arthur Zeitz, was himself one of the fatalities. It was in Cloncurry that the disease fought its rearguard action in the north; 83 cases in 1928 provided the last major outbreak. Hubert Brown went from Brisbane to investigate it bacteriologically.

Typhoid in North Queensland is now a rarity. There has not been a death ascribed to it there for at least nine years. (The cause of death of three residents of Palm Island included in Figure IX for 1953 was para-typhoid.) Among 1700 cases of fever investigated at the Innisfail Field Station in the four years 1951 to 1955, only one somewhat doubtful case of typhoid was recognized.

Here is a major victory over an infectious disease. The achievement of hygienists of the twentieth century in bringing typhoid under control climaxes the achievements of clinicians and bacteriologists of the nineteenth century in defining and understanding it. The story of typhoid is an inspiration and encouragement to those who are engaged in a difficult battle against some obstinate disease.

"O Million-Murdering Death"[1]

The history of malaria in Australia has been reviewed by Cilento and Baldwin (1930) and Ford (1950). Much that was called "malaria" or "fever and ague" in the early days cannot be accepted as malaria. However, Elkington (1912) (Figure X), after a careful investigation, was satisfied that severe forms of malaria had frequently recurred in the north, particularly in the Gulf country. He had no doubt that the epidemic which devastated Burketown was malignant malaria.

FIGURE XI

× = Some localities where malaria has occurred, mostly taken from Cilento and Baldwin (1933); Lucinda Point from Marks (1946); Daintree from personal observation of a benign tertian case in 1933

Precision came into the diagnosis at the beginning of the century. When Richard O'Brien went to practise in Cairns in 1904, he took a microscope with him, and his reports on malaria in 1908 and 1909 are the first in North Queensland to be based on the demonstration of parasites in blood films. Of 173 cases, 114 were benign tertian, 21 quotidian (that is, double benign tertian), 33 quartan and five malignant. They came from Cairns, Yarrabah, Mareeba, Russell River, Normanton and near

[1] In this apostrophe in 1897, Ronald Ross referred to the 1,300,000 who died of malaria every year in India. A recent estimate gives a world total of over two million deaths annually.

Winton. Another seeker after precision was Harold Pridham, who found parasites in the blood of patients in the Innisfail Hospital in 1907–1908 (Heydon, 1927).

Some places where malaria has occurred are shown in Figure XI. They are dotted along the shores of the Gulf and throughout Cape York Peninsula. It is a land of sparse and scattered population, of small or transient mining townships, cattle stations and aboriginal missions, though places like Croydon and the Palmer hold memories of former greatness. Kidston, where a rush followed the discovery of gold in 1907, illustrates how malaria can strike. In 1910, among its population of 400, there were 120 cases with 24 deaths. The local anophelines had become infected with malignant malaria from two miners returning from New Guinea. However, malaria cannot continue in a community unless there is a sufficient concentration of people, for there is no animal reservoir. The freedom of the Gulf in recent years is due, in part at least, to depopulation.

The only well populated centre with a frequent history of malaria is Cairns. A city set amid swamps, it has suffered a series of attacks since 1881, when it was scarcely five years old (Collinson, 1939). Subsequent to O'Brien's report, there was an extensive outbreak in 1913. In 1917, Breinl and Taylor found the blood of 88 persons out of 657 to contain malarial parasites—45 simple tertian and 43 malignant. There was a small outbreak in 1922 and another in 1942, when there were over 600 cases of the benign tertian type. In the latter, Heydon proved that the vector was *A. farauti*. At Cairns was located the Land Headquarters Medical Research Unit, the work of which during the last war led to a profound reduction in the incidence of malaria among Army personnel in New Guinea.

Over the years there had been a gradual filling in of the swamps at Cairns, but the 1942 outbreak stimulated more intensive action. The Army, in conjunction with the Allied Works Council, using modern earth-moving equipment, set out systematically to eradicate the mosquito breeding grounds that for sixty years had menaced Cairns with malaria. It was a notable achievement, although the scheme was not completed. Not only was Cairns freed from malaria and the mosquito burden reduced, but also swamps were reclaimed for habitation.

Since 1946, there has not been a single report of malaria acquired on the mainland of North Queensland, although there was an outbreak of malignant malaria on Murray and Darnley Islands in the Torres Straits in 1952.

Here again is victory, but a qualified and uneasy one. There is a constant risk of reinfection, for North Queensland adjoins one of the most malarious regions in the world. If the drainage scheme in Cairns were to be neglected, or if large centres of population sprang up in the Gulf, malaria could strike again. Our victory is incomplete until it is won also in New Guinea and Indonesia.

THE RIDDLE OF "COASTAL FEVER"

In the eighties, when the tide of settlement turned to the humid coast lands, another phase opened in the pageant of fevers. Joseph Ahearne, of Townsville, wrote in 1890 of his experience half a dozen years before, and he may well have been referring to cases from the Mourilyan-Johnstone area.

> I well remember the shocking sights of prostrate men, stricken to deplorable helplessness by the terrible virulence of pernicious malarious influence, who constantly were received in the earlier times at the hospital here from the parties engaged in clearing the scrubs on the rivers in the north.

In that harrowing description, Ahearne recorded an important epidemiological observation—"clearing the scrubs". We need not accept that all his cases were malarial, but that association of fever with clearing the jungle has been repeatedly confirmed, right down to the present time.

Dyson (1889) gives more detail about these fevers, classifying them as typho-malaria.

> On the Johnstone River in the early days of settlement, before the dense scrub was cut down, malarial fevers were very prevalent, and a considerable number of Europeans first employed died there, or were invalided in consequence; but as the ground became cleared, so the fevers also became a milder type and less prevalent . . . The typho-malarial, observed by Dr. (T. George) White at Geraldton (Innisfail), is decidedly a malignant form of malarial fever, and is decidedly fatal . . . In several cases the illness appeared to come on suddenly, and in others there was a history of neglected intermittent or remittent fever for some time previously . . . Dr. White also says that . . . *bad* cases became as "*yellow as a guinea*" and that he has had three such cases (one European and two Kanakas) which resulted fatally. In each of these, jaundice supervened before death, but it is not the rule, as he did not observe it in others of his cases whether fatal or not . . . The duration of typho-malarial is from two to three weeks, but death may occur as early as the second day.

What were these coastal cases?

That question was to exercise inquiring minds for fifty years. Some cases, no doubt, were malaria; most were certainly not. The vague but useful "typho-malaria" was no longer admissible once the specific nature of typhoid

and malaria was understood. Sixty cases at Mossman in 1907 were reported as *pestis minor*, enlargement of lymph glands suggesting a relation to plague, then present in North Queensland (Annual Report, Commissioner of Public Health, 1906–1907). Herbert Chesson (State Health Officer) and Harry Beardmore (Bacteriologist) investigated the outbreak; the results of their tests for plague were negative.

FIGURE XII

Describers of "Mossman fever". (A) O. Smithson (1910). (B) P. S. Clarke (1913). (C) H. Priestley (1914). (D) J. W. Fielding (1914)

"Filarial fever" had a vogue for some years. However, all attempts to equate the fevers with known infections failed, and new designations came into use—"cane fever", "scrub fever", "coastal fever". The last was still current as recently as 1940.

The first clear recognition that a new entity was present came in 1910 from Oliver Smithson (Figure XII). He named it "Mossman fever", for, he explained, it was in the Mossman district that this peculiar fever was mainly found. In describing the symptoms, he noted that the onset was somewhat sudden, the temperature rising within a few hours to 102°, 103° or even 105° F. The duration of fever was generally from ten to fourteen days. He mentioned the enlargement of lymph glands, but made no reference to a rash.

Fever was certainly common around Mossman. Philip Clarke reported in 1913 that he had seen nearly 1500 cases there in five years. Fortunately, the mortality was quite low—less than 1%. Clarke's description amplifies that of Smithson, but conflicts with it in some respects. He observed the onset, as a rule, to be gradual, the temperature rising to a maximum on or about the tenth day. The fever lasted twenty-one days, and, in 30% of cases, there was an extensive macular rash.

In 1914, Breinl and his assistants spent a month in Mossman to investigate the fever. They found its duration to vary from three days to three weeks and, being impressed by the lymphadenitis, renamed it "endemic glandular fever". In all the cases they observed the patients had a rash. They strove hard to find its cause. They vainly examined smears of blood and of the inflamed lymph glands. They were unable to culture an organism from blood or urine. One success they had: they twice transmitted the infection to monkeys by inoculating them with the blood of a patient.

About this period, complications arose at Sarina, near Mackay. Reports came of serious cases of fever among men working in or near the scrubs to the west of that town. Between 1915 and 1922, there were about 100 cases with 19 deaths (Derrick *et alii*, 1953). There was a general resemblance to "Mossman fever", but the mortality rate was much higher. It was alarming to the community and puzzling to the doctors.

On Stuart Kay (Figure XIII), the forceful Medical Officer of Health of Sarina Shire, fell the responsibility of dealing with it. Acting at first on the presumption that it was typhoid, he cleaned up the sanitation of the area—a highly desirable measure — and protected the inhabitants with typhoid-paratyphoid vaccine. When those failed, he campaigned for scientific investigations, and coopted the aid of the local Member of Parliament. In response to these urgings, Robert Telford, State Health Officer, visited Sarina in 1922 and prepared a comprehensive report. Unfortunately, no copy has survived. At the same time, Hubert Brown set up a laboratory in the sugar mill at Sarina, and gave the final blow to the typhoid hypothesis. Frank Wheatland described "Sarina fever" at the Medical Congress in 1923, and so brought it to the notice of the medical world.

It is quite in accord with the traditions of research that the solution of the problem did not come as a result of these earnest direct efforts. In 1922, "Sarina fever" spontaneously disappeared, and has rarely been seen since. That was probably because clearing the scrub

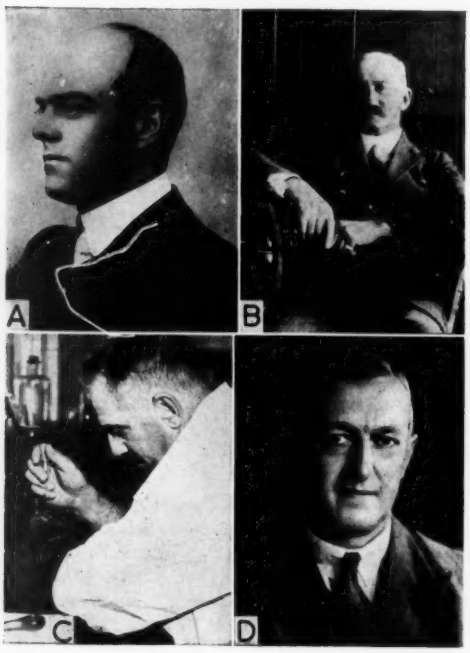

FIGURE XIII

"Sarina fever" personalities: (A) S. Kay, Medical Officer of Health, Sarina Shire. (B) R. W. Telford, State Health Officer, whose comprehensive report was filed. (C) H. E. Brown, who made bacteriological investigations. (D) F. T. Wheatland, Medical Officer, Australian Institute of Tropical Medicine, Townsville, 1922–1923, Medical Officer-in-Charge, Commonwealth Health Laboratory, Toowoomba, 1923–1926, who described "Sarina fever" (1924) and was the first to recognize murine typhus in Queensland

did away with the environment that had sustained the infection. The explanation of its nature was to come from work done elsewhere.

In 1918, Breinl visited Innisfail to make a malaria survey. After finding both benign tertian and malignant types near Mourilyan, he studied fever cases and made a most significant observation. There were two unclassified fever syndromes, not just one. He recognized at Innisfail a few cases of the "endemic glandular fever" he had seen at Mossman, and noted that many of these had previously appeared in the hospital returns as typho-malaria. He also observed seven patients with a shorter type of fever, lasting five to eight days, and with symptoms that did not coincide with locally known fevers. Many of these cases had been diagnosed as malaria.

This insight of Breinl, although it does not appear to have been widely appreciated, clarified the issues and resolved conflicting reports. It was clear that "Mossman fever" was a mixture of fever types; Smithson had emphasized the shorter with rapid onset and Clarke the longer with gradual onset.

Hints as to what those entities might be came from various sources. In 1922, Hone described a series of cases in Adelaide closely resembling typhus, and, in 1926, Wheatland reported similar cases in Toowoomba. As a result of these leads, "endemic typhus" began to be diagnosed on clinical grounds in North Queens-

FIGURE XIV

These established the presence of leptospirosis in Australia: (A) G. C. Morrissey, Medical Superintendent, Ingham Hospital. (B) T. J. P. Cotter, Medical Officer-in-Charge, Commonwealth Health Laboratory, Townsville, 1933–1936. (C) W. C. Sawers, School of Public Health and Tropical Medicine, Sydney. (D) G. F. Lumley, Medical Officer-in-Charge, Commonwealth Health Laboratory, Townsville, 1936–1941, historian of dengue

land. In 1923, Cilento commented on the close resemblance of "endemic glandular fever" to the mite-borne Japanese river fever, and suggested also a possible relation of some cases to "seven-day fever", which had been shown in Japan as far back as 1918 to be a mild form of leptospirosis.

Very informative reports came from Malaya, which, like North Queensland, presented a complex of fever types. In 1926, Fletcher and

FIGURE XV

These identified scrub typhus: (A) R. Y. Mathew, Medical Officer-in-Charge, Commonwealth Health Laboratory, Cairns, 1933–1937. (B) A. M. Langan, Medical Superintendent, Cairns Hospital. (C) M. L. Unwin, Medical Superintendent, Tully Hospital. (D) W. G. Heaslip, National Health and Medical Research Council Fellow, working at Cairns, 1938–1940

Lesslar discovered that Malayan typhus comprised two types—an urban (comparable with that of Hone and Wheatland) and a rural or scrub type. These could be distinguished in the laboratory by the Weil-Felix test. In the urban type, the patient's serum agglutinated *Proteus* X19; in the rural type, *Proteus* XK. Then, in 1928, Fletcher reported that a common cause of fever in Malaya was leptospirosis.

The hunt was now closing in. In 1930, Baldwin prepared a report for circulation among key personnel, drawing together the possible causes of fever in North Queensland, so that when occasion arose appropriate investigations might be made.

The anticipated occasion came in October, 1933, when an outbreak occurred in the cane-fields of Ingham. Gordon Morrissey (1933) (Figure XIV), who practised there, diagnosed leptospirosis clinically and appealed for specific investigations. These were carried out by Timothy Cotter, Medical Officer in charge of the Townsville Health Laboratory, and William Sawers, who came from the School of Public Health and Tropical Medicine, Sydney (Cotter and Sawers, 1934). The leptospiral nature of the cases was soon established, and strains of leptospiræ were cultured from patients and from rats. George Lumley (1937), Cotter's successor at Townsville, reported that the strains were of two types, which he named *australis A* and *australis B*.

With typhus, the occasion found the man when, in 1933, Randolph Mathew (Figure XV) became Medical Officer of the Cairns Laboratory. With the cooperation of Alfred Langan (1935) and Leslie Unwin (1935), Medical Superintendents of the Cairns and Tully Hospitals, he demonstrated serum agglutinins for *Proteus* X19 or *Proteus* XK in a series of fever patients. Typhus in North Queensland was thus shown to include the same two components as in Malaya, scrub typhus being more common than murine (urban). Unwin (1935), in the course of seven years, treated 1500 cases of "coastal fever", the majority of which he considered to be typhus. In some, he noted the presence of the characteristic eschar. Mathew reviewed his many investigations in 1938. Gordon Heaslip, working in Cairns, carried the identification of scrub typhus further (1941). By mouse inoculation, he isolated and demonstrated the rickettsia not only from patients but also from rats and bandicoots. He further concluded that the likely vector in North Queensland was *Trombicula deliensis*.

Since the presence of leptospirosis and typhus was established in North Queensland, knowledge about them has multiplied. Lumley's two types of *Leptospira* have increased to thirteen and, in recent years, leptospirosis has been by far the commonest fever encountered. To Mathew's two types of typhus, a third has been added—tick typhus. A few cases of this mild infection were found by army medical officers among soldiers undergoing jungle training on the Atherton Tableland. In Malaya, Lewthwaite

and Savoor showed that the scrub typhus of Malaya was identical with the river fever of Japan.

Leptospirosis has undoubtedly been a component of " coastal fever " since the earliest days of settlement. White's cases of fatal jaundice at Innisfail could have been either leptospirosis or malaria. The cases of " dengue " in Cairns reported by O'Brien (1908), occurring in a non-epidemic period without the rash and without the third-day remission, were probably leptospirosis, as also was the shorter type of fever observed by Breinl at Innisfail in 1918. Smithson's description of " Mossman fever " is in many respects characteristic of leptospirosis; his Chart 1 is typical. It is curious, however, that Smithson, Clarke and Breinl made no reference to jaundice, which Morrissey found in 13% of his cases of leptospirosis.

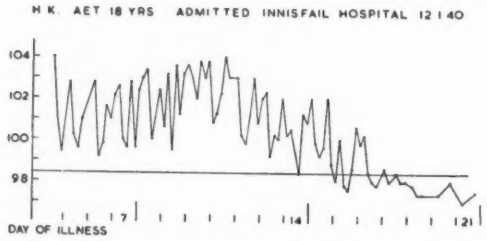

FIGURE XVI

Temperature chart in a case of scrub typhus at Innisfail, 1940. In its course it corresponds exactly to " typho-malaria "—compare Figure VIII

Perhaps the presence of such a striking symptom removed a case from the category of " Mossman fever ".

Scrub typhus must also have been present from the beginning, although no one previous to Unwin reported eschars. It is scrub typhus that is characteristically associated with clearing scrub, and the temperature chart of a proved case from Innisfail in 1940 (Figure XVI) is a perfect example of " typho-malaria ". " Sarina fever " was essentially scrub typhus.

On the other hand, murine typhus was introduced here with imported rats and is spread by their fleas. In recent years, its incidence has increased at Atherton.

Over the years, the heavy mortality of the early days subsided. Scrub became cleared, primitive hardships of living ameliorated and transport to hospital improved, and, more recently, specific therapy has come into use. But the prevention of these diseases has not yet been achieved.

With scrub typhus, a steady stream of cases is still occurring, although there are several useful preventive measures. Clothing can be impregnated with substances like dibutyl phthalate which will kill invading mites. Mites in a particular locality can be greatly reduced by spreading certain chemicals over the ground. The need is to adapt and apply these methods to local situations.

The prevention of leptospirosis is a much more difficult problem. One useful measure has been adopted since the first recognized outbreak at Ingham—that is, the burning of cane before cutting. This is valuable, as far as it goes; it destroys exposed leptospiræ, dries up surface moisture and drives out rats. But it applies only to cane-cutting, and it fails when it is most needed—in wet weather. The use of protective footwear is advocated, but is unpopular. Two obvious approaches—to drain wet fields and to destroy rats—are being employed. But to carry these out on an adequate scale would be a formidable undertaking, and new light on prevention is very desirable.

PERIODIC INVASION

Another fever, dengue, has invaded North Queensland periodically since 1885, and from there has spread to southern Queensland and northern New South Wales. It gives occasion to reflect on the long gap that may separate the acquiring of knowledge and its application. In the 1905 epidemic, T. L. Bancroft in Brisbane put forward strong evidence that dengue was spread by the domestic mosquito, *A. ægypti*, and, in the 1916 epidemic, this was fully established by J. B. Cleland in Sydney. From that date it has been known that dengue can be prevented by suppressing this mosquito, which is a feasible procedure. Indeed, in 1911, Elkington had already begun an attack on it and other mosquitoes. Yet, in 1926 and 1941, epidemics began as before in the north and swept unchecked right down the coast. Partial victory was manifest in the 1953 epidemic; for the first time, the march of dengue was halted, and Brisbane, now free of *A. ægypti*, remained unaffected. That march need never have begun, but, in North Queensland, victory is still elusive.

" THAT WHICH THEY HAVE DONE BUT EARNEST OF THE THINGS THAT THEY SHALL DO "

In reviewing these fevers of North Queensland, we have stood, as it were, on Mt. Bartle Frere, and surveyed the whole landscape. With typhoid, the complete story is spread before

us from the early obscurity, through the successive phases of clinical observation, bacteriological precision and designed control, to final victory. With malaria, we can see a qualified success. With dengue, victory is within our grasp, if we will but take it. With scrub typhus, the means for control are to hand, and the outlook is promising. With leptospirosis, although there have been notable advances, effective control is not in sight.

On the whole, it is an encouraging picture. The victories won sustain the faith that the infections that still distress us will also be overcome.

That is the message of the early physicians who wrestled with " typho-malaria " ; of James, with his clear analysis of " Gulf fever " ; of Goldsmith, who launched the move for medical research and education in our tropics ; of O'Brien, who took a microscope into the fight ; of Frodsham, the shepherd of souls, whose initiative and drive got the Tropical Institute established in Townsville ; of Breinl, a born researcher and master of techniques, who had the gift of going to the heart of a matter ; of Elkington, as he inculcated sanitary righteousness ; of Kay, campaigning for the investigation of the disease he could not comprehend ; of Cilento, whose vision set the northern fevers in their worldwide relationships ; of the others whose contributions have been noted here, and of the many unnamed ; of all who, at the bedside, in laboratory or in health office, recorded their observations and speculations, step by step rolled back the veil of obscurity, strove not only against germ and insect but also against disappointment, ignorance and apathy, and devoted their energies to the health of the community.

To those who fought the good fight, whether or not they saw victory in their time, this address is dedicated.

ACKNOWLEDGEMENTS

The search for early records of fever has been made a pleasure by the willing assistance of the staff of the Library of this Institute, the Queensland Parliamentary Library, the Oxley Library and the Historical Society of Queensland, Mr. J. H. Williams, junior, of Mackay, and Mr. J. W. Collinson. Photographs are reproduced by courtesy of the Registrar of the Medical Board, Professor E. Ford, Professor A. H. Baldwin, Dr. J. Breinl, Dr. M. C. Clarke, Dr. T. J. P. Cotter, Dr. W. G. Heaslip, Dr. W. R. Horsfall, Dr. A. Ivanov, Dr. G. F. Lumley, Dr. R. Y. Mathew, Dr. G. C. Morrissey, Dr. R. E. Richards, Dr. M. L. Unwin, Dr. F. T. Wheatland, the Diocesan Secretary, Townsville, and the Telegraph Newspaper Company (for Figure XIII, C). In the preparation of the illustrations the writer is greatly indebted to Mr. Max Smith. The Queensland Government Statistician kindly supplied typhoid mortality statistics. Much inspiration in the development of the theme was derived from Esther Price's book " Fighting Spotted Fever in the Rockies " (1948), Naegele, Helena, Montana. Three section headings are quoted from Tennyson.

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AN EPIDEMIC OF PRIMARY ATYPICAL PNEUMONIA IN NORTH QUEENSLAND[1]

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SUMMARY

An epidemic of primary atypical pneumonia occurred in the Innisfail-Tully district in the period November, 1954, to March, 1955.

Febrile constitutional symptoms overshadowed the symptoms of pulmonary involvement. However, of 23 patients, all of whom showed radiological evidence of pneumonia, 20 had a cough. Three showed no abnormal physical signs in their chests. The average duration of fever was nine days.

Five patients with similar clinical features showed no X-ray changes. They are considered to form part of the epidemic.

The patients showed no response to penicillin; the clinical impression was formed that the broad-spectrum antibiotics, especially tetracycline, were of value.

Serological tests gave no evidence that *Coxiella burneti* or viruses of the psittacosis group were responsible for the outbreak. Only three patients of the 28 tested developed cold agglutinins; two developed agglutinins against *Streptococcus* MG. Influenza virus could not be isolated from throat washings of seven patients.

More than half of the patients studied were children. The epidemic appeared to reach its peak at the end of December, and the incidence diminished rapidly in January.

Epidemics of primary atypical pneumonia have been described from army camps and from institutions; outbreaks in scattered civilian communities, such as the one described, are uncommon.

THE 372 patients referred for investigation to the Innisfail Field Station of the Queensland Institute of Medical Research between March, 1953, and July, 1954, included none with the syndrome commonly known as "primary atypical pneumonia". This cannot be taken to mean that no cases occurred in the area served by the Field Station, but it does suggest that the incidence of the syndrome was low. It is of some interest, therefore, that an outbreak of a febrile illness associated with clinical and radiological signs of pneumonia occurred in the Innisfail-Tully district in the period November, 1954, to March, 1955. This paper records the clinical and epidemiological features of the epidemic.

CASE HISTORIES

The first patient recognized fell ill at the beginning of November. His history is as follows:

R.W., aged eight years, schoolboy, of Flying Fish Point (four miles from Innisfail), was admitted to the Innisfail District Hospital on November 3, 1954. He had become ill on November 2, with feverishness, headache, abdominal pain and a dry cough. On examination, his temperature was 102·8° F.; he had small, mildly tender lymph glands in both groins; no abnormality could be found in his throat, ears, chest or abdomen; his eyes were clear, and he had no neck stiffness. His urine was normal to ward tests, and his white cell count on November 4 was 6950 per cubic millimetre, with a normal film. His throat appeared injected on November 4, and administration of penicillin was commenced (80,000 units three-hourly). His temperature remained elevated, especially in the evenings (Figure I). On November 9 his cough was more marked, he had scattered râles in both lungs, and chest X-ray examination showed broncho-pneumonia, chiefly in the lower lobe of the right lung,

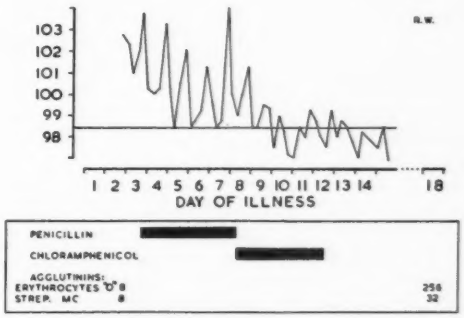

FIGURE I

Temperature chart of R.W.

[1] Received on November 15, 1956.

[2] Senior Research Fellow.

with a confluent area probably situated in the apex of this lobe. Penicillin therapy was ceased, and he was given "Chloromycetin Palmitate", 250 milligrammes four-hourly. On November 11 he had physical signs of consolidation over the right lower lobe; chest X-ray examination (Figure II) showed no

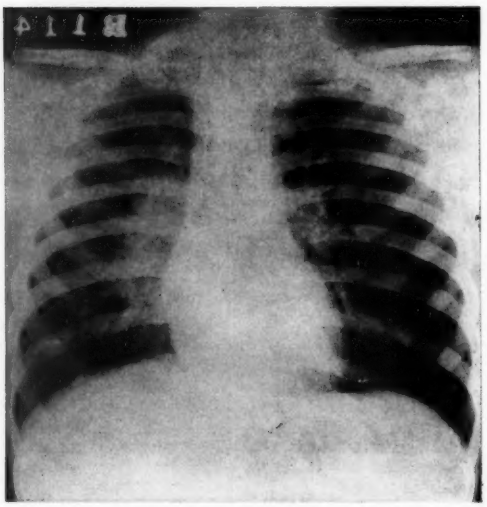

FIGURE II

Chest X-ray film of R.W. on November 11, 1954, the tenth day of illness, showing signs of bronchopneumonia, chiefly in the right lower lobe

significant change in the shadows, but his temperature had fallen to normal. His temperature was slightly elevated on November 12 and 13, but he remained afebrile thereafter. His chest signs subsided slowly, and he was discharged from hospital on November 19.

He developed cold agglutinins and agglutinins to *Streptococcus* MG to diagnostic titres. Other serological tests were negative.

The following patient's course was similar; her lung involvement had a more definite anatomical localization.

H.F., aged ten years, schoolgirl, of Silkwood (25 miles from Innisfail), was admitted to the Innisfail District Hospital on December 24, 1954. She had fallen ill on December 21, with feverishness (especially at night), headache, cough and diarrhœa (following a purgative); she vomited while on the way to hospital. On examination, her temperature was 105° F., her eyes were injected, she was apathetic and drowsy. The admitting medical officer recorded some scattered râles; no other abnormality was found on physical examination. Treatment was commenced with penicillin, 100,000 units three-hourly. On December 26 her temperature showed no response, bronchial breathing and crepitations were heard at her right lung area posteriorly, and chest X-ray examination on the next day (Figure III) showed consolidation involving apical and subapical segments of the upper lobe of the right lung, together with a minor degree of involvement in the left midzone. She was given tetracycline, 250 milligrammes four-hourly; her temperature fell to normal; and chest X-ray examination on December 31 showed some clearing (Figure IV). Examination of paired sera showed no cold agglutinins or agglutinins against *Streptococcus* MG. The results of other serological tests were also negative. Her temperature chart is shown in Figure V.

CLINICAL FEATURES

The clinical features recorded in 23 patients for whom detailed notes were available are shown in the following: feverishness, 23; cough, 20; headache, 17; sore throat, 6; vomiting, 5; sore eyes, 5; abdominal pain, 4; backache, 3; X-ray evidence of consolidation, 23; abnormal chest signs, 20; conjunctival injection, 5; faucial injection, 2; neck stiffness, 2. The average duration of fever was nine days. It must be emphasized that the patients presented as "pyrexias of unknown origin", and that symptoms of pulmonary involvement were always secondary to febrile constitutional symptoms. Cyanosis, dyspnœa, tachypnœa or chest pain was not met with.

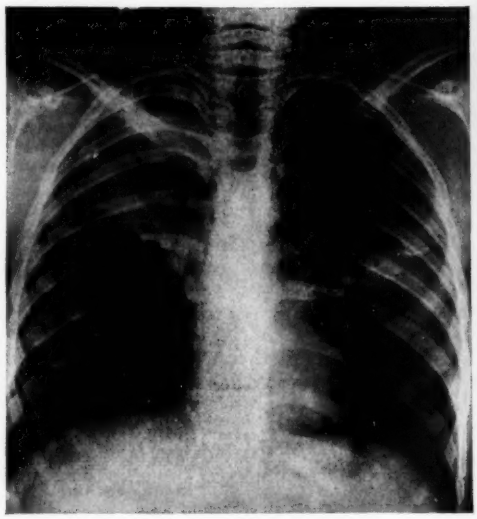

FIGURE III

Chest X-ray film of H.F. on December 27, 1954, the seventh day of illness, showing consolidation in the right upper lobe and slightly in the left midzone. The horizontal fissure is slightly raised and convex upward, suggesting some collapse

Twenty, however, complained of a cough at some time during their illness; three had no cough in hospital, and two of them denied any before admission to hospital; the others could give no history because of language difficulties.

It was usual for cough to be absent or slight on admission, and to become more marked as the illness progressed. Sputum was scanty, and never blood-stained or "rusty".

Six had a sore throat, and several of these had pharyngeal injection. Five complained of sore eyes, and had injected conjunctivæ. Febrile symptoms (feverishness, shivers or sweats) were common; 17 had headache, which was often the presenting complaint. Vomiting, abdominal pain and backache were each mentioned in several cases.

All 23 had consolidation demonstrated by X-ray examination. Abnormal physical signs were found in the chests of 20. A number of these had no abnormalities on admission to hospital, but developed râles and crepitations while in hospital. No râles, or other signs, were heard in three.

White cell counts were carried out on five patients, at various times in their illness; in each case the total and differential counts were within normal limits.

The total duration of illness, from onset to defervescence, varied between four and fourteen

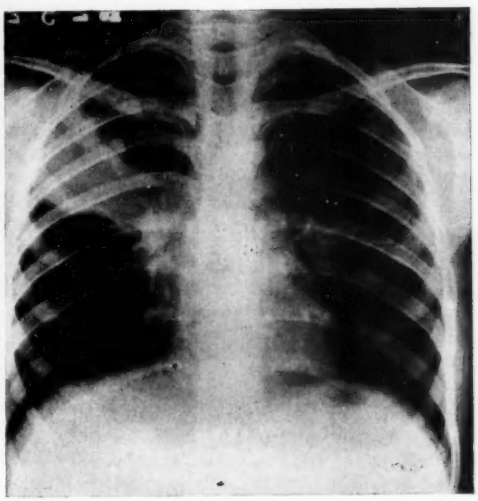

FIGURE IV

Chest X-ray film of H.F. on December 31, 1954, the eleventh day of illness. There has been some clearing in the left midzone and right apex; the right subapical opacity has become denser

days, with an average of nine days. The exact onset date was often difficult to define, as many had vague upper respiratory symptoms for some days before they felt ill. There were some variations in the febrile course; the temperature chart was typically remittent, falling by lysis (Figure I); in some the temperature fell by crisis (Figure V); in some it remained little elevated throughout. The highest temperature recorded was 105° F. Five patients had one or more recordings over 99° F.

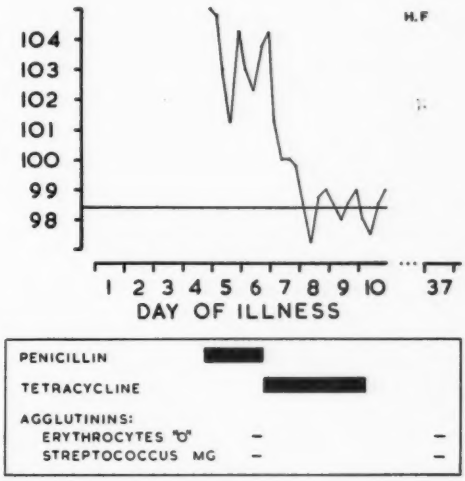

FIGURE V

Temperature chart of H.F.

after an afebrile twenty-four-hour period; in four this rise was short-lived and trivial. The other, a ten-year-old girl, had a six-day period of fever reaching 102° F. after an afebrile period of two days. She developed a rash, and it is likely that the second episode was dengue.

All patients made a complete recovery; none had any complications, and none had any residual pulmonary damage.

Chest X-ray examinations of these patients revealed a number of radiological features. Thus, some showed evidence of areas of segmental collapse; for example, in Figure IV the horizontal fissure is slightly raised and convex upward, suggesting an element of collapse. Serial X-ray films in some cases showed evidence of resolution and of progression occurring together; thus, between Figures III and IV there has been some clearing of the opacity in the left midzone and in the apical segment of the right upper lobe, while the subapical segment has become denser. Two patients had radiological evidence of pleural involvement. The radiological features were considered compatible with the diagnosis of primary atypical pneumonia, but had no

characteristics that would exclude the bacterial pneumonias with certainty.

In addition to the 23 patients discussed, eight patients were admitted to the Innisfail District Hospital during this time with similar X-ray findings and temperature charts; as full clinical details were not available, they have not been included in the series. Their features did not differ from those described.

Five further patients were studied, whose clinical picture suggested that they belonged to the epidemic, but in whom no radiological changes could be demonstrated. All five had a cough, three had a sore throat, four had abnormal chest signs, two had white cell counts performed and findings were within normal limits. Their average duration of fever was nine days. None developed cold agglutinins; they also yielded negative results to other serological tests. It is possible that more frequent X-ray examinations would have clarified their diagnosis; the earlier peribronchial involvement described by American workers (Commission on Acute Respiratory Diseases, 1946) was in some cases detected only by oblique views, which were not performed in the present cases. On the other hand, epidemics of primary atypical pneumonia have frequently been associated with a high incidence of undifferentiated respiratory tract infections (for example, in the experimentally infected volunteers studied by the Commission, *loco citato*).

TREATMENT

The value of the broad-spectrum antibiotics, especially chlortetracycline, in the treatment of patients with primary atypical pneumonia is supported by a considerable body of literature (Schoenbach and Bryer, 1949; Finland *et alii*, 1949; Meiklejohn *et alii*, 1954). All these authors comment on the difficulty in assessing therapeutic agents for a disease which has no specific diagnostic test, has often a vague onset, and is liable to spontaneous recovery without treatment.

A variety of therapeutic regimes were employed in the present series, and the results of treatment do little to confirm or refute previous claims. Most patients received penicillin, either as the only treatment or as a prelude to other treatment, without any obvious response. Nine received penicillin alone; their total duration of fever averaged 8·1 days. Nine received tetracycline, with an average duration of 7·7 days. Eight received chloramphenicol or chlortetracycline, with an average duration of 8·2 days. There is thus no significant difference between the three groups.

It must be pointed out, however, that the patients with severer effects were selected for treatment with the broad-spectrum antibiotics. The rapid defervescence seen in some of these (Figure V) was striking, and was not seen in any given penicillin alone. The general clinical opinion was that tetracycline at least was of value.

LABORATORY INVESTIGATION

Blood was taken on admission to hospital, and at least once during convalescence, from the 23 patients with radiological changes whose clinical features have been discussed, and from the five patients mentioned who had no abnormality demonstrated by X-ray examination. It was allowed to clot at room temperature, and the serum obtained was tested for antibodies against the following antigens: the 13 North Queensland serotypes of leptospiræ, *Proteus* OX19, *Proteus* OXK, *Coxiella burneti*, *Rickettsia mooseri*, *Salmonella typhosa*, *paratyphi* and *schottmülleri*, *Brucella abortus*, erythrocytes "O" (cold hæmagglutinins), *Streptococcus* MG, psittacosis group complement-fixing antigen (prepared from the virus of enzootic abortion of ewes (Dane, 1955)).

The positive findings are listed in Table I. Only three of the 28 were shown to develop cold agglutinins, and one of these reached a titre of only 1 : 16. Two patients developed agglutinins to *Streptococcus* MG; one of these reached a titre of only 1 : 8, which would not be accepted as significant. Thus, even by the most liberal standards only four patients had serological evidence of primary atypical pneumonia.

The results of tests for other agents that may cause pneumonia (those of "Q" fever and psittacosis) were negative, although one patient had evidence of an old *Coxiella burneti* infection. Three developed antibodies against *Proteus* OX19, one against *Proteus* OXK, and one against *Rickettsia mooseri*. In each the titres were low; it seems possible that they represent non-specific serological reactions described in this syndrome (Thomas *et alii*, 1943).

Blood taken from two patients was inoculated into mice intraperitoneally, and a number of blind passages were made. No agent was isolated. Two intracerebral mouse passages were made in one case, with negative results.

Throat washings were obtained on admission to hospital from seven patients and submitted to the Queensland Institute of Medical Research,

TABLE I

Positive Serological Results in 28 Cases

Patient	Day of Illness	Serological Test[1]						Extent of X-ray Involvement
		Cold Agglutinins	*Streptococcus* MG	*Proteus* OX19	*Proteus* OXK	*Rickettsia mooseri*	*Coxiella burneti*	
R.W.	2	8	8	–	–	–	–	Right hilum and lower lobe
	18	256	32	–	–	–	–	
M.R.	5	–	–	32	–	–	–	Left hilum and pectoral segment. Left upper lobe
	13	64	–	64	–	–	–	
L.F.	3	–	–	–	–	–	–	Left lower lobe
	63	16	–	–	–	–	–	
J.C.	2	–	–	–	–	–	–	Left midzone
	13	–	–	–	–	–	–	
	30	–	8	–	–	–	–	
P.T.	8	–	–	–	–	–	–	Apical segment of right lower lobe
	22	–	–	16	–	–	–	
V.C.	10	–	–	32	–	–	–	Right upper lobe
	29	–	–	32	–	–	–	
I.N.	3	–	–	–	16	–	–	Left lower lobe
	43	–	–	–	16	–	–	
S.Z.	2	–	–	–	–	–	–	Nil
	78	–	–	–	–	8	–	
L.V.	4	–	–	–	–	–	8	Right lower lobe
	12	–	–	–	–	–	8	
	22	–	–	–	–	–	8	

[1] Figures in test columns represent reciprocals of titres. " – " indicates that the result was negative.

Brisbane, where they were examined by Miss I. A. Brown and Mr. J. G. Carley. No virus, however, was obtained on amniotic inoculation of embryonated eggs.

EPIDEMIOLOGY

The 36 patients mentioned in the discussion of clinical features form a homogeneous group for study. Their basic data can be analysed as follows.

Age Incidence.—Patients' ages ranged from three years to fifty-three years. The highest incidence (13 cases) was in the ten to fourteen years age group. Seven were under ten years of age.

Sex.—Thirteen were male and 23 female.

Occupation.—The majority were school children. Among older patients there was no suggestion that any occupational group suffered a higher incidence than the general population.

Geographical Distribution. — Seventeen patients came from the town of Innisfail, and the remainder from the townships in its district. Only one was recognized in the town of Tully. Two brothers from Flying Fish Point included the first patient diagnosed. Five patients from Silkwood included the last patient accepted as part of the epidemic.

Time Incidence.—The first patient fell ill on November 2, 1954, the last on March 4, 1955; the latter may have represented a sporadic case, as the previous case had occurred on January 12. Six were recognized in November, 20 in December, nine in January and one in March. Twenty fell ill in the period December 20 to January 12. The epidemic appeared to reach its peak just before New Year's Day. This may have been artificial; as the epidemic progressed, and the nature of the disease was recognized, more patients were treated in their own homes. It is estimated that only about half the patients treated reached hospital. No definite correlation can be found between time incidence and geographical distribution. Thus cases occurred in Silkwood in December, January and March.

Family Contacts.—There were a number of instances in which more than one case occurred in a family, or in groups of people in close contact. In several they were widely apart (for example, two brothers who fell ill on November 2 and December 27). In others the patients fell ill together (a brother and sister on December 5 and December 6). Others are of greater interest: two brothers fell ill on December 20 and January 4, a girl and her mother on December 14 and January 3, and two children living in adjacent flats on November 12 and November 22. Another child from the same flats fell ill on December 26. Thus cases in contact occurred after intervals

of ten to twenty days. The incubation period of primary atypical pneumonia is commonly accepted as fourteen to twenty-one days (Horsfall, 1952).

DISCUSSION

In the absence of a specific laboratory test, the diagnosis of primary atypical pneumonia depends on clinical criteria. These have been listed as follows (Schoenbach and Bryer, 1949): gradual onset, with non-productive cough, absence of initial chill and bloody sputum, X-ray changes out of proportion to the physical signs elicited, normal white cell count, normal bacterial flora in sputum and throat washings, no evidence of other recognized viral or rickettsial agents, failure of penicillin or sulphonamides, and the development of cold and *Streptococcus* MG agglutinins in a proportion of patients.

The present series meets most of these requirements, although no bacteriological investigations were carried out on sputum or throat washings. The clinical features closely resemble those described in previous accounts of primary atypical pneumonia (Commission on Acute Respiratory Diseases, 1946; Dingle, 1951). Some support is lent by the proportion (admittedly low) of patients who developed cold agglutinins. While individual cases of bacterial pneumonia due to penicillin-resistant organisms may have been included in error, it seems unlikely that the epidemic as a whole could be explained in this way.

The name "primary atypical pneumonia" has come under attack in recent years. Scadding (1948) described the term as "not only meaningless but confusing". Crofton (1952) suggested that "pneumonia associated with cold agglutinins" or "pneumonia associated with agglutinins to *Streptococcus* MG" would be more suitable. Dingle (1951) concluded that "'primary atypical pneumonia' is the most widely and generally used (term) today". This ruling has been followed in the present paper.

The present epidemic has several features of epidemiological importance, in the scattered civilian population affected and the evidence of case to case contact. Primary atypical pneumonia usually occurs in endemic form, although small epidemics have been described (Horsfall, 1952). These epidemics have usually occurred in army camps or in institutions (Snyder *et alii*, 1952). Dingle (1951) mentions that case to case spread is rarely apparent in sporadic cases, but may be during epidemics. Jordan (1949) observed 19 families in which multiple cases of primary atypical pneumonia and associated respiratory infections occurred.

This outbreak did not coincide with the influx of cane-cutters and tourists which occurs between May and September each year. It appeared to be localized to the Innisfail-Tully area; no cases were encountered at Babinda, 20 miles north, and Base Hospitals at Cairns and Townsville did not record any increase in incidence. It is of some interest that a number of cases were seen in the Babinda-Cairns area in May, 1955, in some of which cold agglutinins were found by Dr. W. R. Horsfall, of the Commonwealth Health Laboratory, Cairns.

The outbreak may perhaps be explained by postulating the introduction of an infective agent which is endemic in the larger cities, producing only sporadic cases among members of urban populations who are largely immune, into a comparatively isolated area, where a low level of immunity permitted it to assume epidemic form.

ACKNOWLEDGEMENTS

All the serological tests were performed by the Laboratory of Microbiology and Pathology, Department of Health and Home Affairs, Brisbane. The Institute of Medical and Veterinary Science, Adelaide, supplied the psittacosis group complement-fixing antigen. Attempts at isolation of influenza virus in eggs were made by the Queensland Institute of Medical Research. The author is indebted to Mrs. M. Macgregor, Librarian of the Queensland Institute of Medical Research, for much help with references, to the practitioners of Innisfail and Tully for their cooperation, to Dr. E. W. Abrahams, Director of Tuberculosis in the Department of Health and Home Affairs, who commented on the X-ray films, and whose opinions have been quoted in the paper, and to the Medical Superintendent of the Innisfail District Hospital for permission to publish the case histories.

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STUDIES OF PLATELET FUNCTION AS A GUIDE TO THE SEVERITY OF THROMBOCYTOPENIC PURPURA AND THE POSSIBLE MECHANISM OF PURPURA AND HÆMORRHAGE[1]

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Summary

Abnormal results from 121 tests of platelet thromboplastic function were recorded in 30 patients with thrombocytopenic purpura of various types. The presence and degree of hæmorrhage or purpura, and the platelet count were also recorded at the time the platelet function tests were performed. The results showed that when the platelet thromboplastic function was below 12%, all patients were frankly bleeding; when it was between 12% and 25%, some patients were hæmorrhagic, while others showed purpura only; when it was above 25%, virtually nc patients were hæmorrhagic. Purpura occurred over the range 25% to 53% platelet thromboplastic function. There was very little correlation between the degree of hæmorrhage and platelet function, and between platelet numbers and the occurrence of purpura and hæmorrhage.

It is thought that purpura is a manifestation of vascular endothelial damage, but that hæmorrhage follows only when the coagulation mechanism is severely deranged by platelet dysfunction.

The test of platelet thromboplastic function has proved useful in the assessment of the severity of thrombocytopenic purpura and in predicting the occurrence of hæmorrhage.

At the present time there is no satisfactory single method of assessing the severity of thrombocytopenic purpura, of gauging the response to therapy, or of following the progress of chronic cases. In fact, the clinical state of the patient gives a much better indication than any laboratory test. The platelet count is useful in the diagnosis of the disease, in that it is reduced in thrombocytopenic purpura; but it may not rise during a clinical remission, although lasting remissions in this disease are usually accompanied by an eventual return to normal numbers of platelets. However, the platelet count is quite dissociated from the severity of hæmorrhagic manifestations (Bonnin, 1956; Wald and McAuley, 1955). In fact, Craddock, Adams, Perry and Lawrence (1955) have shown that the platelets in the normal dog can be depleted to extremely low levels without the occurrence of any hæmorrhagic manifestations, confirming the previous findings of Brill and Rosenthal (1923), Roskam (1929) and Thompson, Richter and Edsall (1934).

Stefanini and Dameshek (1955) have cited the dissociation between platelet numbers and hæmorrhagic manifestations as evidence of the important part played by vascular damage in these diseases. It is the purpose of the present paper (i) to support this concept but to demonstrate the equally important part played by platelet dysfunction, (ii) to demonstrate the usefulness of the study of platelet thromboplastic function in the assessment of the severity of the disease, and (iii) to suggest a possible mechanism of purpura and hæmorrhage, which may be related but separate phenomena.

[1] Received on January 8, 1957.

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Materials and Methods

The Patients Studied

Thirty patients have been examined in this study, all of whom showed a thrombocytopenia of varying degree and had a platelet thromboplastic function reduced below 81% when compared against normal platelets yielding between 133% and 150% thromboplastin. They include all patients studied on whom satisfactory clinical and laboratory observations have been made, and consist of 11 patients with acute myeloid leuchæmia, four with the amegakaryocytic (aplastic) type of thrombocytopenic purpura and 15 with the megakaryocytic type. The megakaryocytic group is comprised of nine patients with idiopathic thrombocytopenic purpura and six with a similar condition associated with systemic *lupus erythematosus* (two patients), quinidine sensitivity (one patient), pregnancy (two

patients) and liver disease (one patient, who refused complete investigation). The great majority of the patients have been adults, and only three children are included, because it is often unwise or very difficult in young children to obtain the 20 or 30 millilitres of blood which are necessary to provide sufficient platelets for the tests in thrombocytopenic subjects.

Platelet Function Tests

The application of the thromboplastin generation test (Biggs and Douglas, 1953) to the study of platelet thromboplastic function has been fully described elsewhere (Bonnin, 1956). Briefly, the technique is as follows.

Platelet Suspensions.—Siliconized glassware is used throughout. An appropriate volume of venous blood (usually 18 millilitres) is withdrawn and mixed with one-ninth volume (usually two millilitres) of 3·8% sodium citrate solution. The whole is centrifuged in an M.S.E. major centrifuge (a refrigerated centrifuge is used in hot weather only) at 800 to 1000 revolutions per minute for ten minutes, the low-spun (platelet-rich) plasma is obtained and direct platelet counts are made. The volume of the plasma is measured as it is pipetted into a centrifuge tube. This plasma is then centrifuged at 3000 revolutions per minute for fifteen minutes, and the button of platelets is washed three times in normal saline solution. A film of a plastic non-wettable substance is placed over the top of the centrifuge tube, and the tube is shaken vigorously to resuspend the platelets in between washings. The original volume of low-spun plasma and its platelet content being known, the washed platelets are finally resuspended in a volume of saline calculated to yield a suspension of 1,600,000 platelets per cubic millimetre. A similar suspension containing 1,600,000 normal platelets per cubic millimetre is prepared at the same time. The platelets of each tube are emulsified with separate wooden probes to liberate the platelet factors before the final suspensions in saline are made.

The Thromboplastin Generation Tests.—The normal platelet suspension is tested by the thromboplastin generation test (Biggs and Douglas, 1953) using normal serum diluted one in ten and normal alumina plasma. The patient's platelet suspension is then compared against the normal control platelets, but subsampling of the thromboplastin generation mixture may have to be continued beyond six minutes (up to nine or ten minutes) to obtain the maximum yield of thromboplastin when the platelet function is abnormal. The patient's serum and alumina plasma are also tested for possible defects. The occurrence of a serum thromboplastic defect in some of the more severe cases of thrombocytopenic purpura has been described previously (Bonnin, 1957). The fact that this and the platelet thromboplastic defect are cumulative and the importance of measuring the combined thromboplastic deficiency have been emphasized.

The Recording of Purpura and Hæmorrhage

Some difficulty arises in recording accurately the appearance and subsidence of fresh purpuric lesions in patients whose skins already show heavy purpuric manifestations, since the purpuric spots take some days to fade. The mucous membrane of the mouth, tongue and palate, however, have nearly always been amongst the earliest sites involved, and fine purpuric lesions will disappear within twenty-four hours after the process is checked. Even larger hæmorrhagic bullæ will show obvious signs of regression in twenty-four hours and will disappear completely in two to four days after a patient undergoes a remission. Where it has not been possible to obtain quantitative information from the skin, the absence or obvious regression of purpuric lesions in the mouth have been recorded as " — ", the first appearance of less than ten fresh minute purpuric spots as " ± ", and the appearance of larger and more numerous lesions as " + ". Mild degrees of frank bleeding from any one site have been recorded as " ++ ", bleeding from more than one site as " +++ " and severe hæmorrhage from several sites as " ++++ ". Substantial ecchymoses have been regarded as " ++ " hæmorrhage and multiple mild bruising as " + " purpura. In this series of patients, frank bleeding has occurred from the nose, gums, kidneys and bowel (melæna), and into the *fundus oculi*, the brain and the submucous and subcutaneous tissues. It is unfortunately the case that, even with careful observations, this method of recording purpura and hæmorrhage can only be approximate.

RESULTS

In most of the cases of acute leuchæmia and the megakarocytic type of thrombocytopenic purpura, there has been a rapid return to normal of the platelet thromboplastic function coincidently with a clinical remission in response to ACTH or cortisone therapy. For this reason, it has been possible to obtain only between one and four abnormal results of tests per patient. In the amegakaryocytic patients, however, many abnormal results of

tests and observations on the hæmorrhagic state have been obtained. The results of 121 platelet thromboplastic function tests, expressed in terms of the percentage of thromboplastin generated, have been tabulated according to whether the patients showed frank hæmorrhage and purpura, purpura alone without hæmorrhage or neither purpura nor hæmorrhage at the times when blood samples were taken for the tests. The results are shown graphically in Figure I.

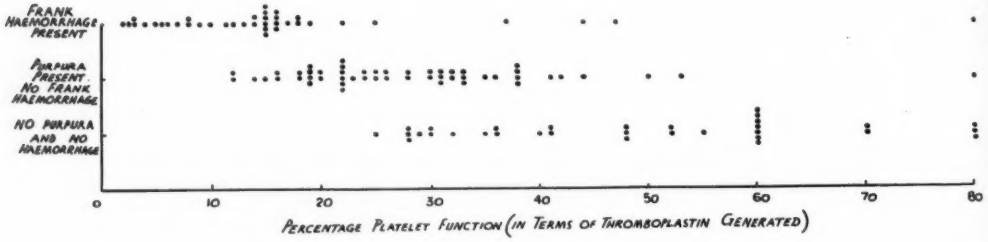

FIGURE I

The relationship of hæmorrhage and purpura to platelet thromboplastic function

It can be seen that hæmorrhage occurred in all patients on every occasion when the platelet thromboplastic function was below 12%. Within the range 12% to 25%, 20 readings were obtained when frank bleeding was present and 25 readings were recorded when the patients showed purpura but no frank hæmorrhage. This range may therefore be considered as the range over which frank hæmorrhage is liable to occur. Above the level of 25% of platelet thromboplastic function, only four tests were recorded when hæmorrhage was present. Three of these were consecutive tests performed during the terminal stages of a patient with aplastic anæmia who had contracted a *Clostridium welchii* infection. Other tests on this patient also showed uncontrollable intestinal bleeding at platelet function levels of over 100%, and it was obvious that some different mechanism was operative during the last few days of life. Prior to this, many readings had been obtained from which it had been possible to predict the hæmorrhagic state of the patient with some degree of accuracy. These readings have therefore been disregarded as far as the uncomplicated association between platelet function and hæmorrhage is concerned. The fourth reading above 25% and associated with hæmorrhage from the gingivæ occurred in a pregnant woman, and the two pregnant women with thrombocytopenic purpura who have been studied have both shown other inconsistencies which suggest that the mechanism of the association between platelet function and hæmorrhage is complicated by other factors during pregnancy. For instance, this hæmorrhage occurred without any associated purpura, and such a finding is rare without further complicating factors unless the onset is sudden and the symptoms are severe with a very low platelet thromboplastic function. In these cases frank bleeding may be the presenting sign of thrombocytopenic purpura and may not be preceded by purpuric lesions, but this finding has been unusual.

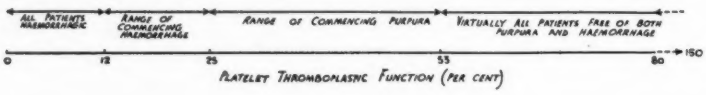

FIGURE II

The diagrammatic representation of the relationship between hæmorrhagic manifestations and platelet thromboplastic function

Between the values of 25% and 53% of platelet thromboplastic function, 25 readings were recorded in purpuric patients and 19 in patients who were neither purpuric nor hæmorrhagic. This range may therefore be considered as that over which purpura is liable to occur. Above the level of 53% no patient was ever purpuric or hæmorrhagic with the exception of the one patient with aplastic anæmia mentioned above.

In general, these results show that platelet thromboplastic function studies can be used

quantitatively to assess the hæmorrhagic state of the patient as is illustrated in Figure II.

The severity of hæmorrhage was recorded according to the scheme described above and is correlated with the platelet thromboplastic function in Figure III. There is only a very approximate relationship between these two

DISCUSSION

The unmodified thromboplastin generation test was not designed to give quantitative results. However, for the estimation of platelet thromboplastic function, it can give results which are sufficiently accurate and reproducible for clinical purposes if certain precautions are observed.

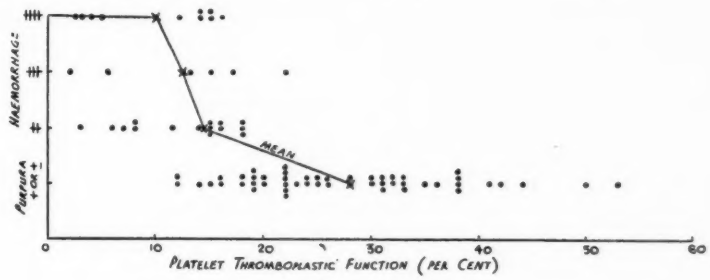

FIGURE III

The relationship between the severity of hæmorrhagic manifestations and platelet thromboplastic function

data as recorded; but when the mean values of the platelet thromboplastic function tests are considered in relation to the severity of hæmorrhage in uncomplicated cases (Figure III), it would appear that the method of recording is sufficiently accurate to show that hæmorrhage is more severe as the platelet thromboplastic function is reduced. The quantitative recording of purpura as described above is too inaccurate to be of value when compared against the platelet thromboplastic function, and both degrees of purpura are recorded together.

Direct platelet counts were made on the majority of whole blood specimens collected for the platelet function tests. It is of interest to show in Figure IV that there is no definite relationship between frank hæmorrhage and purpura on the one hand, and platelet numbers on the other.

For progressive estimations in following the course of patients with chronic forms of thrombocytopenic purpura, the same normal subject has been bled as far as possible to supply the normal reagents for the tests. Thus, a patient's platelet function can be compared successively against that of the same normal platelets, and variations in the activity of reagents in the normal serum and normal alumina plasma should be reasonably well standardized. In no case have normal reagents been used unless a clotting time of between eight and nine seconds of one sample of the substrate plasma has been obtained (133% to 150% of thromboplastin generated). If a platelet suspension calculated to contain 1,600,000 platelets per cubic millimetre (Bonnin, 1956) is used, variations of up to 100,000 platelets per cubic millimetre will not appreciably alter the yield of thrombo-plastin.

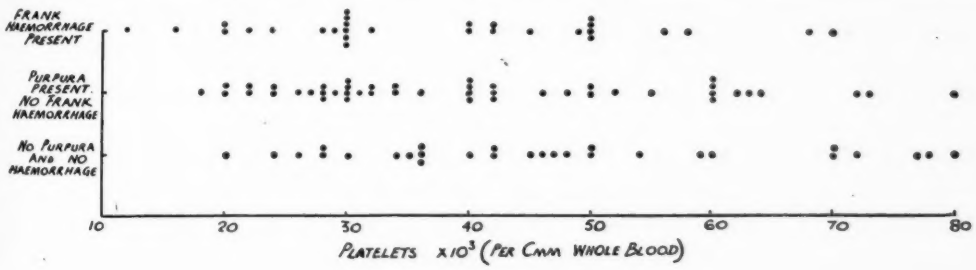

FIGURE IV

The hæmorrhagic state of patients compared with the platelet count

Apart from any errors inherent in the test, there is a considerable error in correlating the platelet function with the hæmorrhagic state of the patient caused by the method of recording purpura and hæmorrhage quantitatively. One of the chief reasons for the lack of close correlation in Figure III between the degree of hæmorrhage and platelet function is the time factor. Many of these readings were recorded in patients who had been given the minimal amount of ACTH or cortisone which would keep them above the level at which frank hæmorrhage was liable to occur. When the platelet function dipped below the hæmorrhagic level, it was promptly brought up again by increased dosage before serious hæmorrhage had time to develop. In individual case studies where there has been little or no response to these drugs, it has been apparent that when a patient is left with a platelet thromboplastic function below the hæmorrhagic level, the degree of hæmorrhage becomes progressively more severe with time at the same level of platelet function. For this reason the correlation between platelet thromboplastic function and the hæmorrhagic state of the patient might have been more accurate if only untreated patients had been considered. There would then be fewer readings of patients with purpura only, occurring in " the range of commencing hæmorrhage ", and relatively more with frank bleeding.

In general, hæmostasis may be considered to depend upon three main factors: (i) the efficiency of the vascular mechanism, including the ability of damaged vessels to contract (Macfarlane, 1941) and the effects of serotonin; (ii) the degree of damage to the vascular endothelium; (iii) the effectiveness of the coagulation mechanism. The last two factors are of extreme importance in the occurrence of spontaneous hæmorrhages in thrombocytopenic purpura. The importance of the vascular lesions has been emphasized by Duke (1912), Bedson (1922), Ackroyd (1949*a*) and many others. Bedson (1922) showed that an anti-platelet serum produced damage to the vascular endothelium in addition to lysis of platelets. Again, Ackroyd (1949*a*) has demonstrated the independent damage to the capillaries and platelets produced by the same antigenic complex in sedormid purpura and has drawn attention to the antigenic similarity of these two tissues (Ackroyd, 1949*b*). This author (1949*a*) has indicated that the main lesion in purpura is the capillary permeability, and that the deficiency in platelets is of secondary importance. Although platelets seemed to be associated in some way with the occurrence of hæmorrhage, it was not clear whether the thrombocytopenia played any important part.

The present work would suggest that a reduction in platelet numbers is not the most important factor. Providing there is sufficient vascular damage, the occurrence of frank bleeding seems to be directly related to the degree of platelet thromboplastic dysfunction or, where there is an additional serum thromboplastic defect, to the state of the coagulation mechanism as measured by the total thromboplastic efficiency. Assuming that the same factor produces both vascular and platelet functional defects, both would be affected together, and the degree of each would be roughly proportional. The platelet function test would then give an approximate estimation of both the degree of vascular damage and the state of the coagulation mechanism. When the former is sufficiently severe to produce a purpuric leak, obvious platelet dysfunction can be detected by the thromboplastin generation test; but because one is not dependent upon the other, there is a relatively wide range of platelet thromboplastic function over which purpura may occur (Figure II). It is therefore assumed, in common with the above authors, that purpura is a manifestation of capillary damage only. When the coagulation mechanism is not impaired, the lesion is repaired, and blood flow is again established through an intact vessel, leaving a purpuric spot or small ecchymotic area, depending upon the degree of vascular damage.

When the platelet thromboplastic function (or total thromboplastic efficiency) is so reduced that the coagulation mechanism is severely impaired, then the capillary leaks can no longer be repaired, blood continues to flow through the damaged vessels, and frank bleeding results. Frank hæmorrhage, therefore, would depend not only upon vascular damage but directly upon the platelet thromboplastic function, accounting for the much narrower range of function over which frank hæmorrhage will occur (Figure II).

No doubt the above hypothesis is over-simplified, and it may be far more accurate to allow for each variable (platelet thromboplastic function, vascular damage and platelet numbers) independently. Also, many platelet functions may be deranged. Stacey, in a publication by Hardisty and Wolff (1955), has shown that the mean platelet serotonin content is greatly reduced, together with platelet thromboplastic function in hæmorrhagic thrombocythæmia. Unless these factors were reduced propor-

tionally with platelet thromboplastic function, they would not have been allowed for in the above test. That the vascular mechanism is deranged also in thrombocytopenic purpura is suggested by the prolonged bleeding time which can be demonstrated at platelet function levels above those at which spontaneous hæmorrhage is liable to occur. However, the vascular mechanism is possibly more concerned with the severity and control of hæmorrhage rather than with its occurrence, and this is possibly why hæmorrhage, once commenced, seems to continue at platelet function levels higher than those at which it is liable to occur. In spite of its imperfections, the study of platelet thromboplastic function has proved to be a useful method of assessing the severity of this disorder and of predicting the danger of severe hæmorrhage.

The relationship between hæmorrhage and platelet function is present only in the absence of other complicating factors. For instance, excessive menstrual bleeding has not been included in this series because a local complicating hæmorrhagic mechanism is active at these periods. Menorrhagia requiring transfusion has occurred in one patient with chronic idiopathic thrombocytopenic purpura when the platelet thromboplastic function has been well above the expected level of spontaneous hæmorrhage. Similarly, patients may bruise and bleed after trauma when there is no spontaneous hæmorrhage. The terminal intestinal hæmorrhage which is referred to above in the patient with aplastic anæmia and which showed no relation whatever to platelet function has been considered to result from some complicating hæmorrhagic mechanism. It is interesting to note that this patient had then received continuous ACTH or cortisone therapy in large doses for 188 days and had many of the clinical signs of Cushing's syndrome. Hæmorrhage associated with Cushing's syndrome is well known, and Thorn *et alii* (1953) have drawn attention to the massive hæmorrhage which may occur when these drugs are administered to patients with peptic ulcers. Prunty (1956) described a patient with Cushing's syndrome who died from uncontrollable gastric bleeding, and drew attention to the gastric hæmorrhage which may occur in patients undergoing treatment with cortisone. It may well be that these drugs themselves have a direct or indirect detrimental effect on vascular endothelium if given in large doses for prolonged periods. Their beneficial action seems to lie in the protection of the vascular endothelium and platelets against the damaging action of the serum or plasma factor present in thrombocytopenic purpura.

It has been assumed that the serum anti-platelet-thromboplastic factor present in thrombocytopenic purpura is also responsible for the vascular endothelial damage, but the factor or factors responsible for the morphological changes in the megakaryocytes and the reduction in platelet numbers has not been considered. There is now abundant evidence to suggest that capillary resistance and platelet thromboplastic function are quite dissociated from the platelet count, and it is likely that at least two separate factors are responsible. The nature and action of platelet agglutinins are still not certain, and they cannot be demonstrated in the blood of all patients. A platelet functional defect, however, has been present in every thrombocytopenic patient showing purpura or hæmorrhage of the type under consideration. One is at once attracted by the analogy of the red cell antibodies where different types exist, such as the complete or saline-agglutinating antibodies, the blocking or albumin-agglutinating antibodies and the sensitizing antibodies which are responsible for positive antiglobulin reactions. Not all of these various types are necessarily demonstrable together, and a similar state of affairs may well be present in the factors concerned in thrombocytopenic purpura. It may be that the platelet function test is the most sensitive method of detecting the effects of immunological reactions involving platelets, just as the direct Coombs test is one of the most sensitive and sometimes the only method of demonstrating involvement of erythrocytes.

Thrombocytopenia alone does not necessarily imply the presence of an immunological reaction of the type under consideration. Two patients have been examined at this laboratory who have both shown pronounced erythroid and megakaryocytic hypoplasia in the bone marrow. Each has had repeated platelet counts of between 30,000 and 50,000 platelets per cubic millimetre, and one patient has now had this condition for nearly two years. Neither patient has ever had purpura or a hæmorrhagic episode, and the results of platelet function studies on each patient have been repeatedly normal.

ACKNOWLEDGEMENTS

I am most grateful to Professor H. N. Robson for his stimulating interest in this work and for his criticism of the manuscript. I am indebted to Miss Maureen Jenner and Mr. E. Wyld for their able technical assistance.

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THE HISTOLOGY OF GENERALIZED PULMONARY EMPHYSEMA

II. Diffuse Emphysema[1]

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Summary

The evolution of morphological changes in the air passages from the early focal lesions of emphysema to those associated with the clinical disease is presented.

Progress of the focal lesions leads to involvement of the whole of the secondary lobule, at which stage the emphysema is no longer focal but " diffuse ". This diffuse change occurs at different stages of development of the focal lesions in different lungs ; the morphological evolution of both early and late change is described along with some of the more common intermediate forms.

Observations supporting the hypothesis of pathogenesis of emphysema presented in Part I of this paper have been made, and the hypothesis is extended to account for diffuse change. These diffuse lesions are aggravated by bronchospasm and, relatively late in the morphological evolution of the disease, by expiratory collapse of bronchioles. The emphysematous changes themselves, in turn, aggravate this last factor.

The hypothesis of pathogenesis is reviewed, and morphological evidence is correlated with several aspects of physiological observations on alterations of lung function in the disease.

The relation of chronic bronchitis to emphysema is analysed in view of these observations, and it is concluded that chronic bronchitis cannot be said to " cause " emphysema, but, rather, both are related by way of the common factor of inflammatory disease of the bronchioles.

If you be not too much cloyed with fat meat,
our humble author will continue the story.

Second Part of *King Henry IV* :
Epilogue.

The descriptive terminology of pulmonary emphysema is as abundant as it is vague. However, from the morphological viewpoint the term " focal " applied by Heppleston (1947) to lesions restricted to the middle of secondary lobules is eminently acceptable, since it is accurate and readily defined. Changes affecting the whole lobule are distinct from focal lesions, and in this presentation, therefore, have been termed " diffuse ".

In the preceding paper (McLean, 1957*b*) the earliest recognized histological lesions of emphysema were focal ; it is proposed to show that diffuse emphysema does not arise *de novo*, but develops from these focal lesions. In some examples, " diffuse change " was observed to occur at an early stage of development of the focal lesion ; and although, in others, the process of dilatation of the air spaces in the middle of lobules often progressed to a remarkable extent without significant change in peripheral passages, nevertheless, macroscopically it was evident that with further evolution of these well-developed focal lesions the whole lobule was eventually involved. Thus " diffuse change " occurred at different stages of development of the focal lesion ; and, although intermediate forms were common, it is convenient to describe two types of lesions—early diffuse change at a stage where the focal lesion was just visible macroscopically and late diffuse change due to further evolution of well-developed macroscopic focal emphysema.

These alterations in the pattern of the lesions usually occurred first in one part of the lung, most often in the upper lobes, while much of the lung still exhibited focal lesions. At this early stage of diffuse change, the patients from whom the lungs were taken gave no history of symptoms relevant to emphysema, except perhaps those with the most advanced generalized focal lesions. Therefore, description of the evolution of these lesions has been broadly divided again into pre-clinical and clinical stages.

[1] Received on August 27, 1956.

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PRE-CLINICAL EMPHYSEMA

Early Diffuse Change

Morphological evidence of diffuse change was found in some lungs early in the evolution of the focal lesion and was evidenced by extension of the process of dilatation and disruption of the alveolar walls enclosing passages leading from the middle of the lobule. The characteristics of the focal lesion in which this change occurred have been described (McLean, 1957*b*), and there the lack of black pigment and old inflammatory damage in the walls of the emphysematous spaces seen in these lesions was considered to account for the different pattern of the emphysema in such lobules when compared with well-developed focal lesions.

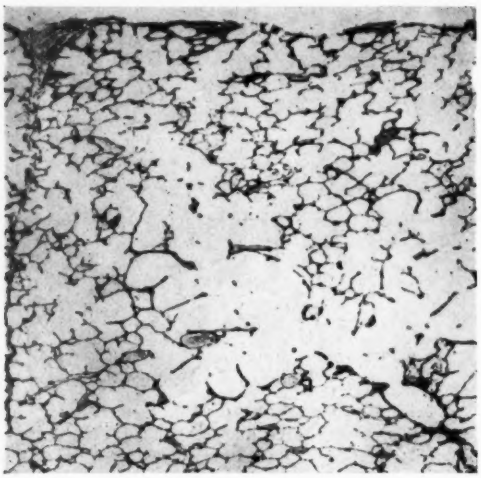

FIGURE I

Photomicrograph showing a corner of a secondary lobule—outlined by the pleura (above) and a septum (left). The most peripheral passages are normal. Dilatation and disruption of passages can be seen in the branches of a second order respiratory bronchiole (lower right); of these, alveolar ducts are most affected. (×22)

In the early lesions, which were essentially focal, the passages most affected by dilatation and disruption of their walls were usually second and third order respiratory bronchioles; a few very early lesions showed the greatest change in the alveolar ducts, but in these at least the third order of respiratory bronchioles was also affected to some extent (Figure I). Progress of the emphysematous change within a lobule resulted not only in dilatation of peripheral passages and disruption of alveoli in their walls, but also in further disruption in the middle of the lobule; this led to the formation of a maze of intercommunicating spaces referred to in the previous paper (McLean, 1957*b*) as a " common pool ".

With spread of the process of disruption of alveolar walls in passages leading from the common pool to the periphery, the pool extended until all the air spaces in the lobule intercommunicated by innumerable pathways. In those examples in which diffuse change had occurred particularly early, the central dilated spaces were usually just visible macroscopically; at the stage where, histologically, the pool had extended to the periphery of the lobule, much of the tissue was simply a mesh consisting of a number of bared strands of elastic tissue occasionally connected by the walls of shallow alveoli.

At this stage the interlobular septa were usually tenuous and were often not readily recognized. With further progress of the lesion, septa became disrupted at a few points, so that free communications between lobules were seen (Figure II). Later, this disruption became so complete that only thicker septa remained, and most of these were incomplete; this could be appreciated more readily macroscopically than in sections (McLean, 1956*a*). It progressed until, at the stage at which it was evident that the emphysema had been associated

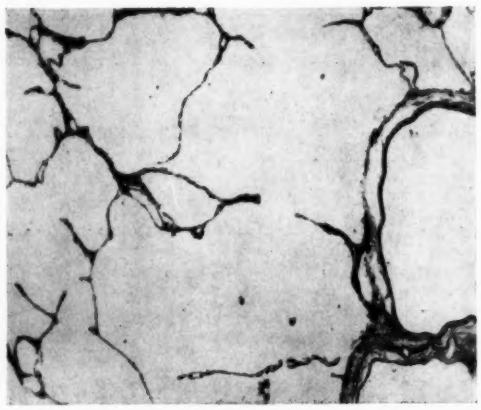

FIGURE II

Photomicrograph showing a thin interlobular septum (from upper left) joining a thicker septum (lower right) in which there is a vein. Near the vein there is a defect in the septum by which adjacent emphysematous spaces communicate. In serial sections this defect was found to be approximately circular, with rounded edges. (×60)

with significant dyspnœa during life, the lobular pattern of the lungs could not be recognized, and the "common pool" was often of segmental size or larger. This type corresponded to the "fine-strand" variety of generalized diffuse emphysema described macroscopically (McLean, 1956*a*).

Concurrently with the development of the common pool there was increasing evidence of bronchiolar damage. The bronchiolar changes associated with the initial lesion (McLean, 1957*b*) became more obvious with the progress of the emphysematous change. Even in the earliest lesions, bronchioles opened relatively abruptly into the common pool, and, in some examples, communications between bronchioles and distorted air spaces included apparently simple defects in the walls of bronchioles—even two or three divisions proximal to their final branches. Although not common, some of these defects resembled the secondary communications formed in normal lung, but the possibility that they were original passages could not be completely excluded (Figure III).

With increasing diffuse change the small bronchioles showed progressive evidence of loss of muscle. They were usually thin-walled,

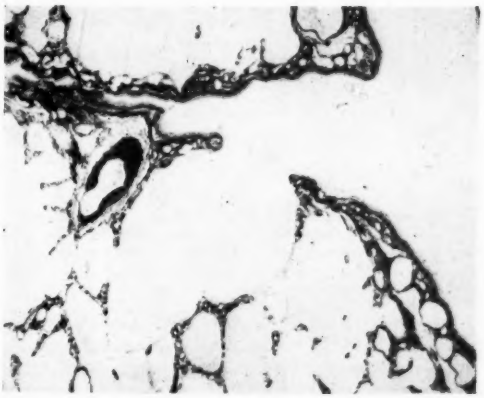

FIGURE III

Photomicrograph showing, near the bifurcation of a bronchiole, a defect in wall structures by which the bronchiole communicates with adjacent emphysematous passages. In the absence of this defect the affected bronchiole would have been termed "preterminal"; the communication was thought to be secondarily formed. Elastin stain. (×22)

and the elastic tissue of their walls was often normal, but the subepithelial net was sometimes widened and occasionally even duplicated. In more advanced emphysema of this type, bronchioles of all sizes often had little smooth muscle over lengths of their walls and were usually somewhat dilated where this loss was greatest.

Obliteration of bronchioles, the importance of which was emphasized previously, was by no means obvious in the early stages. Partial obliteration was seen only occasionally; and

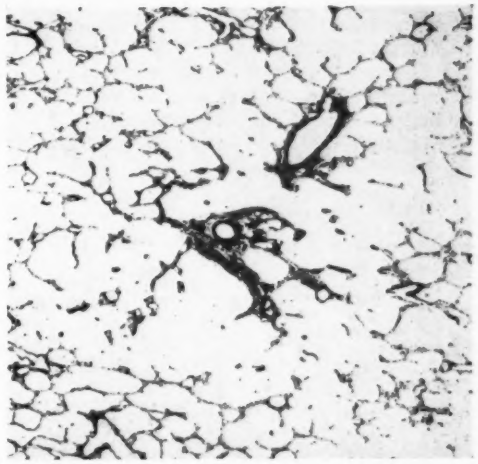

FIGURE IV

Photomicrograph showing typical "early diffuse change" in passages adjacent to respiratory bronchiolar divisions. A mass of musculo-elastic tissue leads peripherally (immediately left of the central artery) from the wall of the respiratory bronchiole to end among spaces which are aerated only indirectly. Elastin stain. (×22)

then only in the minimal form of a thin layer of connective tissue between subepithelial elastic net and epithelium. Although complete obliteration was never positively recognized in early lesions, some dilated spaces contained masses of musculo-elastic tissue in their walls but were supplied by what appeared to be distorted alveolar ducts. The musculo-elastic tissue in such examples was continuous with that found in non-epithelialized scar tissue lining damaged respiratory bronchioles (Figure IV). Thus, though not completely proven, obliteration of a respiratory bronchiole was strongly suggested.

With greater alteration in the passages forming the pool, the order of remaining passages could no longer be identified, so that this method of tentatively identifying obliterated passages became inapplicable.

Many of the features of the early diffuse lesion can be illustrated by a somewhat more

detailed consideration of an example in which a secondary lobule (with parts of those adjacent) was serially sectioned and reconstructed in detail. Diffuse change was minimal and had occurred at a somewhat later stage of the development of focal emphysema than that already described; there was a small amount of black pigment in the walls of some of the passages. (Unfortunately, in this case there was much desquamation of epithelium owing to the fact that fixation was delayed some ten hours. Fixative injected endobronchially had therefore carried debris, including loose epithelium, into the peripheral passages. It communicated with the surrounding air spaces. This bronchiole (*A* in Figures VI and VII) did not divide (although normally three to five divisions would be expected) and finally opened into a typical "common pool" in intercommunicating dilated spaces, from which relatively unchanged passages led to the periphery of the lobule (Figure VIII).

However, proximal to this common pool a passage (*B* in Figures VI and VII) was observed to arise from an epithelium-lined recess in the midst of the aggregation of scar tissue in the outer wall of the bronchiole (Figure VB). The lumen of this passage became larger as it

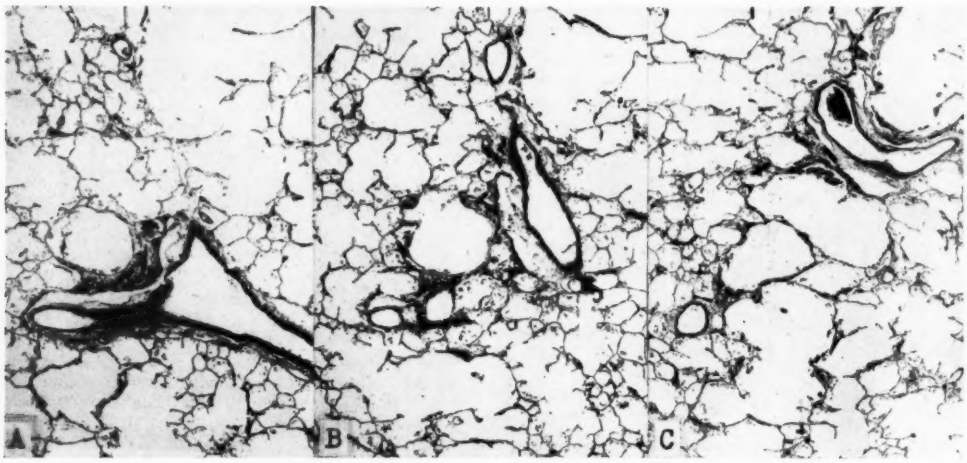

FIGURE V

Photomicrograph showing three sections at approximately 150μ intervals through the "bronchiole" supplying half of a secondary lobule in which there was early diffuse change. In the first section the bronchiole shows some pigmented scar tissue in its wall on one side. More peripherally it becomes less distinct; beyond the third section a typical "common pool" was formed. (×12)

will also be noted that the arteries are relatively undilated compared with those fixed by the intravenous route, as in Figures I and II.)

The portion to be described was definitely not less than one-half of a secondary lobule. This secondary lobule was identified by reconstruction of the surrounding lung and its bronchiolar supply and by identifying the remnants of septa. The branch of the lobular bronchiole supplying this portion was most abnormal; the wall near the arterial branches was thick, and the subepithelial tissue was collagenous and contained a moderate amount of black pigment. There was no muscle and little elastic tissue in the wall at this site (Figure V). On the other side the wall was unusually thin, and at one site the bronchiole was traced peripherally until it also ended by opening into the common pool. A diagrammatic reconstruction showed the complexity of the communications in the region of the pool (Figure VI). A reconstruction of the same area in which only the vessels and scar tissue were represented (Figure VII) suggested the possible fate of the bronchiolar divisions that had disappeared. Neither the "bronchiole" A nor the passage B, in view of what was concluded in the preceding paper (McLean, 1957*b*), were necessarily original passages, but any explanation of the distribution of the scar tissue that did not include the possibility of bronchiolar obliteration was not consistent with the alterations that had demonstrably occurred in the air passages.

Moreover, although this reconstruction cannot be said to afford definite evidence of obliteration of small bronchioles, the findings were consistent with observations made on the evolution of

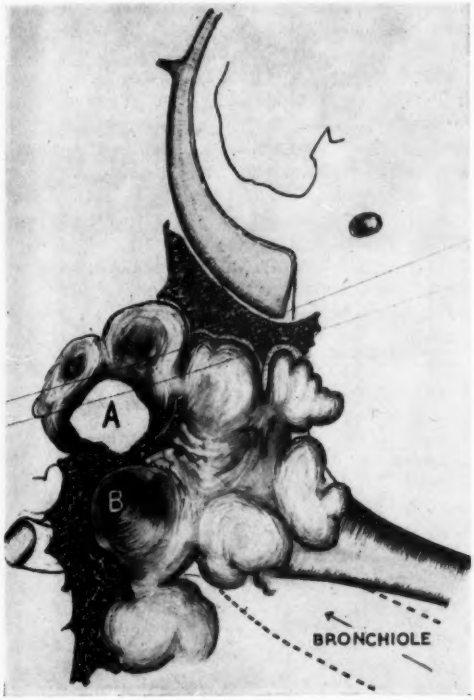

FIGURE VI

Diagram showing, in three-dimensional form, the relation of bronchiole A to the blindly arising passage B. Farther peripherally there were numerous other communications between A and B

obliterative changes and their recognition in old lesions (McLean, 1957*a*).

Similar reconstruction of more severely affected lung demonstrated only that bronchiolar damage increased with the severity of the emphysema; because of the gross alteration in architecture, at this stage, it was not feasible to obtain evidence even suggesting direct relation between bronchiolar obliteration and dilatation and disruption of more distal passages.

Late Diffuse Change

Diffuse change often did not become apparent until the focal lesion was well developed. The passages towards the periphery of the lobule were unaffected in typical macroscopic focal emphysema, but in many lungs showing typical focal change elsewhere there was, in some areas, complete involvement of lobules. The peripheral passages in such areas not only were disrupted (as in early diffuse change) but also were frequently compressed to some extent, particularly in those relatively rare examples in which, in areas of gross diffuse change, the original centrilobular pattern was still discernible.

In most examples, though both disruption and compression could be seen in the same tissue, compression was not prominent (Figure IX). In these, diffuse change was just apparent histologically at a stage where the lesion macroscopically appeared to be purely centrolobular, the focal lesion being readily recognized

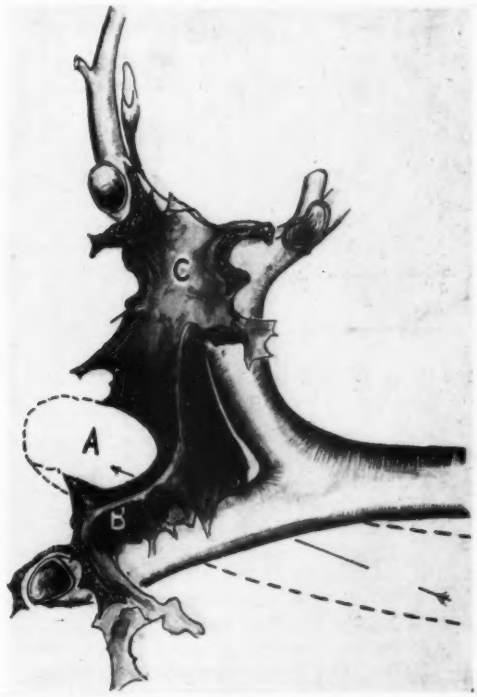

FIGURE VII

Diagram showing only the scar tissue (somewhat exaggerated) and the arterial branches of Figures V and VI. The possibility that remnants of obliterated bronchioles were included in this scar tissue is strongly suggested, for instance, in the region of C

in sections as large confluent spaces related to arterioles. Since the interlobular septa had often been disrupted at this stage, the limits of the secondary lobules could only be inferred

from the vascular pattern, aided at times by the survival of parts of somewhat thicker septa (Figure IX).

Where septa remained, it was often clear that the focal change had progressed, so that only a few irregular air spaces remained between

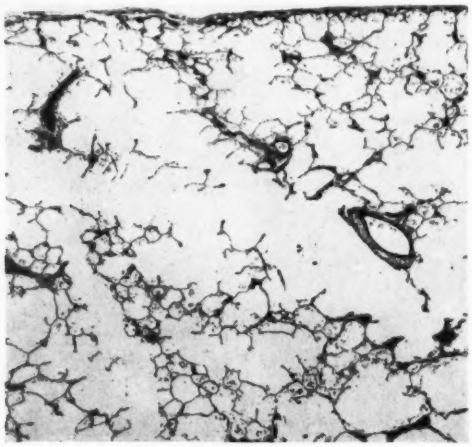

FIGURE VIII

Photomicrograph showing, arising from the "common pool" peripheral to Figures VI and VII, a passage of relatively unchanged structure with its attendant arterial branch. (×16)

the "focus" and the septum demarcating the periphery of the lobule (Figure IX). At other sites this process had extended, and "foci" had become confluent (Figure IX).

Those lungs in which compression of the peripheral air spaces was more obvious sometimes showed patchy thickening of septa, but in many areas the septa were disrupted and foci had become confluent.

The communications of bronchioles with the dilated spaces of the "foci" were essentially similar to those in macroscopic focal emphysema, except that evidence of inflammatory damage was more obvious, and bronchioles were often more abnormal. Some bronchioles entered spaces by somewhat tortuous passages, which were occasionally lined by flattened epithelium on one side or over the whole wall. Towards their terminations bizarre structures, such as diverticula or solid cords of epithelial cells, were seen, the cords extending from the bronchiolar lining and ending either blindly (Figure X) or on the surface of an air space.

The walls of small bronchioles always showed damage. It was unusual to find more than occasional groups of muscle cells in the most distal divisions, and the walls were often thickened, consisting mostly of vascular connective tissue containing aggregations of black pigment. Although elastic tissue was usually much reduced in amount, often sufficient survived to indicate that partial organization of the lumen was relatively common. Most black pigment was found in the outer layer of walls, but connective tissue internal to remnants of the elastic tissue net sometimes contained considerable amounts. Such connective tissue arises by organization of material occluding the lumen in acute bronchiolitis (McLean, 1957*a*). Acute bronchiolitis occurring in these emphysematous lungs was commonly seen as a terminal event, and plugs occluding bronchioles sometimes contained, among the polymorphonuclear cells, pigment-filled macrophages; a bronchiole completely occluded

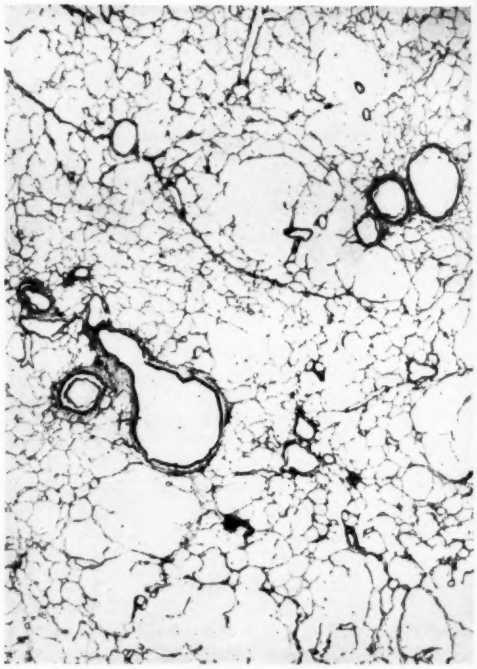

FIGURE IX

Photomicrograph showing an imperfect septum separating two areas each supplied by separate bronchioles. The emphysema, although superficially distributed at random, can be seen to surround arterial branches and is therefore essentially "focal". However, diffuse change is also present, since the focal areas of emphysema almost abut on the septum (above) and are also confluent (below). Extension has occurred more by disruption than by compression. (×5)

by such macrophages was illustrated previously (McLean, 1957*b*). In more normal lungs isolated pigment-filled macrophages were seen only occasionally in exudate plugs.

Reconstruction of a block of tissue showing late diffuse change (one section of which is illustrated in Figure IX) demonstrated that most bronchioles entering the common pool were only three to five divisions distal to a terminal bronchus—a very much reduced arborization compared with the shortest found in normal lungs. Extensive bronchiolar damage began two or three divisions distal to terminal bronchi (Figure XII), increasing peripherally, and also the surrounding air spaces were greatly deformed. Therefore, although bronchiolar obliteration must have been considerable, recognition of the original structures could not be effected. Perhaps further improvements of technique would allow such recognition.

Larger bronchioles were altered to a lesser extent, but usually showed evidence of old damage, such as loss of muscle and increased connective tissue, as did many of the smallest bronchi. The large bronchi and trachea, in this case, were structurally normal, but in other lungs showing this degree of emphysema, even in cases in which there was no acute terminal infection, these passages often showed a general increase in the number of goblet cells in the mucosa and some hypertrophy of the mucus-secreting elements of the bronchial glands. Usually, this change was most obvious in bronchi leading to those areas in which the emphysema was most advanced.

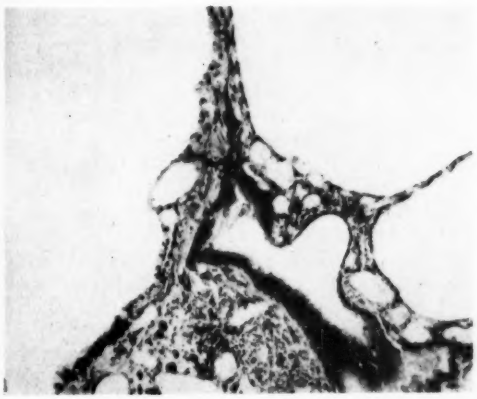

FIGURE X

Photomicrograph showing a distorted bronchiole which, in nearby sections, opened into a large emphysematous space (left). A diverticulum lined by flattened epithelium and a cord of cells extending into adjacent connective tissue are shown; the wall of the bronchiole contains little muscle. Bronchial capillaries are prominent. (×65)

CLINICAL EMPHYSEMA

Some of the emphysematous lungs that were examined were from patients who had been regarded clinically as suffering from emphysema and in whom autopsy had revealed no other cause for their symptoms. These lungs showed gross structural changes and included a small number in which focal lesions were still apparent through the lung; in most there was extensive diffuse change, and, of these, two general types were recognized (with numerous intermediate forms). The first type, in which the diffuse lesion was relatively uniform and unpigmented, and residual strands of tissue were relatively fine, was regarded as an advanced form of early diffuse change. In the more common second type, focal lesions were usually still obvious in much of the lung, but, at other sites (usually in the upper lobe), there was gross emphysema; often there was a less affected rim of lung tissue on the periphery of the lobe. Pigmentation was usual, and the residual strands were generally coarser than in the first type. This second type was regarded as evolving from focal lesions by late diffuse change. The macroscopic features of these two types of lesions have been presented previously in greater detail (McLean, 1956*a*).

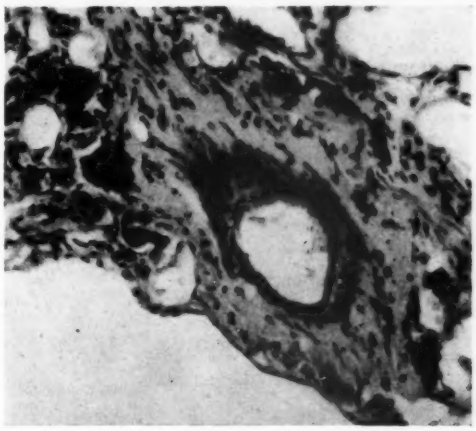

FIGURE XI

Photomicrograph showing a small bronchiole, on division proximal to a "common pool". A few smooth muscle fibres remain in the wall, which is largely fibrosed. (×180)

Histological examination of these lungs was relatively unrewarding, but both types shared certain features. The common pool extended by disruption of septa to include subsegments, then segments and, in severe disease, even whole lobes; in extreme cases this was obvious macroscopically (McLean, 1956*a*). Similarly, bronchiolar damage was greater than in preclinical emphysema, and communications with the common pool were sometimes associated with strikingly abnormal structures of undetermined origin (Figure XII). Usually, many

FIGURE XII

Photomicrograph showing a bronchiole (above) opening into a huge common pool of at least two segments in extent in a grossly emphysematous lobe. On one side there is a mass of black pigment (which also contained some elastic fibres). The associated artery shows considerable intimal thickening. Elastin stain. (×90)

branches of the same bronchiole opened into one huge space, and gross reduction of the number of divisions arising from a first order bronchiole was easily appreciated. Surviving bronchioles sometimes were thick-walled, but more often were thin-walled and obviously dilated; such ectatic bronchioles were particularly common in the "fine-strand", lightly pigmented lungs.

At this stage of evolution of emphysema it was obvious macroscopically and histologically that much lung tissue had been destroyed; it would be unrealistic to suppose that the amount of collagenous tissue present in the lung represented the remnants of all the structures that had originally been present. The conclusion that much scar tissue had been resorbed would appear irrefutable.

Obliterative bronchiolar changes were not often directly demonstrated in advanced emphysema, but, occasionally, recent complete obliteration was seen; partial obliteration producing significant stenosis was not common.

The bronchi usually showed some evidence of old inflammatory damage; loss of muscle was generally obvious only in small bronchi in severely affected parts of lungs. A small number of the patients had died suddenly without terminal acute respiratory infections; some of them had had a chronic productive cough for years, and their bronchi usually showed obvious hyperplasia of the mucus-secreting elements of the bronchi and trachea—the goblet cells of the epithelium and the bronchial glands. By contrast, the large bronchi and trachea of a small group of patients in whom a chronic cough had not been complained of during life were essentially normal histologically. Thus, evidence of persistently hyperactive mucus-production, regarded by Reid (1954) as the earliest morphological evidence of chronic bronchitis, was sometimes not present even in this advanced morphological stage of emphysema (and was never found in non-asthmatics in the early stages of the focal lesion).

A phenomenon, seen only in advanced emphysema, was observed in lungs fixed in the cadaver by intravenous injection. In normal lungs and in lungs showing early emphysema that were fixed by this method, small bronchioles (in areas not showing recent changes), though sometimes contracted, always remained patent; but in areas of advanced emphysema they were often collapsed and flattened, and the lumen was obliterated (Figure XIII). In some cases the lungs were removed from the body soon after the injection (within six hours), and blocks of lung tissue were cut; from these air was removed by applying a vacuum pump to a bottle containing these blocks immersed in fixative. During this process of evacuation it was evident that air escaped relatively slowly from blocks of emphysematous lung, and little of this air escaped from the cut ends of bronchioles; after this process, in which the tissue may be said to be fixed "in forceful expiration", collapse of bronchioles was even more obvious and widespread. On the other hand, with endobronchial fixation of excised lungs, in which the tissue is fixed "in inspiration", this phenomenon was not observed.

The material examined was reviewed in an attempt to find a morphological explanation for the observation that diffuse change was usually seen first in the upper lobes. At the earliest signs of the diffuse change, the basic focal lesion was uniform throughout the lung; only with much further advanced diffuse change was it possible to state that there was greater evidence of bronchiolar damage in the upper than in the lower lobes. The explanation would therefore not appear to be due primarily to greater bronchiolar disease in the upper lobe than in the lower.

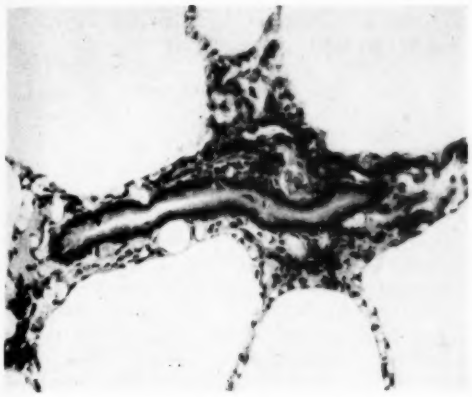

FIGURE XIII

Photomicrograph showing a collapsed small bronchiole (cut transversely) in a case of clinical emphysema. These lungs were fixed in the cadaver by intravenous injection, and most small bronchioles in the more emphysematous regions were similarly collapsed. This "collapse" of bronchioles is regarded as being similar to that occurring in life with expiration. (×90)

It was also hoped to find some hint why the clinical disease occurs predominantly in males. Although too few examples of each sex were examined at the different stages for satisfactory statistical assessment, it was clear, in general terms, that the earliest macroscopic lesions were found at an earlier age in males, and that subsequent progress of the disease was more rapid than in females. Few males aged fifty to sixty years did not have at least early macroscopic generalized focal lesions, whereas several females over seventy years of age had macroscopically normal lungs. Insufficient information on the patients' occupational history and smoking habits was available for indisputable correlation with these findings, and thus the cause of the difference in the sexes remained obscure.

DISCUSSION

An hypothesis of pathogenesis of the early lesions of emphysema was presented in the previous paper, in which these lesions were to be regarded as sequelæ of attacks of acute bronchiolitis. Acute bronchiolitis, the essential lesion in most pulmonary inflammations, is characterized by occlusion of the lumen by a plug which usually contains bacteria from the nasopharynx. If the plug is retained sufficiently long, proliferation of organisms results in a pyogenic bacterial inflammation; the longer the duration of this phase, regardless of the severity of the damage caused by the viral or chemical agent which so often initiates these infections, the greater the likelihood of significant bronchiolar damage and of permanent bronchiolar obliteration.

In the common forms of acute bronchiolitis the plugs extend distally only into the respiratory bronchioles, and the aeration of more distal passages is maintained by collateral ventilation; so that their collapse or consolidation is relatively uncommon, and the clinical diagnosis is usually "upper respiratory tract infection" or "acute bronchitis".

During the acute phase, particularly where many adjacent lobules are affected, air-trapping occurs in these collaterally ventilated areas. The pressure in the passages rises considerably with coughing; this may or may not expel the plugs. If it does not, the pressure, which is maximal in the passages furthest removed from the source of the collaterally supplied air, that is in the passages just beyond the plugs, exerts a disruptive force on their walls. In this manner acute focal emphysema is produced.

Chronic focal emphysema is the result of a number of such incidents, particularly of those in which the acute bronchiolitis has been severe or prolonged. Air-trapping often persists beyond the exudative phase because of permanent obliteration of the arborizations of small bronchioles; this bronchiolar obliteration is a significant factor in the progress of emphysema from the earliest stage at which breakdown of the walls of respiratory bronchioles and alveolar ducts was first recognizable, with formation of what have been termed "common pools".

With the disruption and breakdown of walls of the small air passages, communications between adjacent units increase in number. Thus, in the early stages, this process results in easy ingress and egress to an area which has its normal air supply cut off. With involvement of more bronchioles and further

secondary changes, however, the pathway of the air to the common pool becomes longer and more tortuous. Further air-trapping results in more distension and breakdown of walls. With repetition of this sequence, eventually the pattern of air-trapping and therefore of the emphysema changes. In early lesions emphysema is restricted to the middle of lobules, and the peripheral passages in the lobule are normal, collateral ventilation being sufficiently free to prevent significant air-trapping in these passages. It has been observed that diffuse lesions, in which the whole lobule is involved, were first seen as groups of affected lobules. This might be anticipated because of the free collateral ventilation between lobules that exists in this form of the disease ; only when air trapping in one group of lobules is considerably greater than in adjacent groups would significantly different expiratory pressure gradients exist and disruption of passages follow. At this stage air-trapping becomes regional rather than intralobular.

Two factors could induce this difference in the degree of air-trapping between two regions : increased bronchiolar damage in the affected area or decreased collateral ventilation. In generalized emphysema bronchiolar damage is often relatively uniform throughout the lung, and, in the early diffuse lesions, there is no pathological cause for local reduction of collateral ventilation between lobules. For these reasons the early diffuse changes appear in regions, such as the borders of lobes, where the two surfaces are sharply angled, and at corners in which, for anatomical reasons, collateral ventilation is limited.

In advanced focal emphysema, with increasing bronchiolar damage, the periphery of the lobule eventually becomes involved. In examples where the focal change was particularly well developed, diffuse change was often seen first deep in the lung near the spatial centre of bronchopulmonary segments ; only later does the subpleural region become involved. It is suggested that near the spatial centre of segments, where relatively more space is occupied by vessels and bronchi, the space-occupying effect of the focal lesions interferes more with collateral ventilation of the periphery of the lobules than it does subpleurally.

The development of the diffuse lesion is probably also influenced by other factors. In parts of lungs in which diffuse change was relatively advanced, collapse and flattening of bronchioles was seen in tissue fixed by the intravenous method (particularly when the residual air was rapidly evacuated soon after fixation before processing). With this form of fixation, fluid (including residual air) flows out of the bronchial tree, and the tissue is fixed " in expiration " ; with endobronchial fixation (in which collapse of bronchioles was not seen), the tissue is fixed " in inspiration ". Since collapse was seen neither in normal lungs nor in early focal emphysema, this phenomenon is regarded as analogous to the expiratory collapse of bronchioles inferred to occur in clinical emphysema, as a result of study of the mechanics of respiration in this condition, by von Neergaard and Wirz (1927), Dayman (1951) and many others more recently. The same phenomenon has been demonstrated directly in emphysematous patients by bronchography by Di Rienzo (1949). The most obvious factor producing expiratory bronchiolar collapse in emphysema is the widespread air-trapping that occurs in this condition.

The effects of this phenomenon on the subsequent course of emphysema are twofold. It will increase air-trapping in severely emphysematous parts of the lung and so directly aggravate the condition ; but, probably more significantly, it will also decrease considerably the efficacy of cough in ejecting any material occluding bronchioles, so that acute inflammation of the bronchioles leading to the affected region will be prolonged and more damage result. It is obvious that at this stage of the disease a vicious circle is present, and the disease will advance rapidly with each subsequent infective episode.

Although Di Rienzo (1949) showed that bronchioles within normal lungs could collapse with the violent expiration of coughing, it must be emphasized that this would not induce disruption of the walls of small passages, since the effect throughout the lung would be uniform and no pressure gradients could develop across alveolar walls despite the elevated intra-thoracic pressure. Such pressure gradients would eventuate only when bronchioles remained collapsed in one part of the lung longer than another (that is, when air-trapping in one part was previously greater than in another). Thus expiratory collapse of bronchioles can aggravate established emphysema, but does not produce change in normal lung.

Similar considerations apply to generalized bronchospasm, such as occurs in bronchial asthma. The theoretical conclusion that bronchospasm alone does not ordinarily produce emphysema is supported by the observation of Gough (1952), which has been personally

confirmed, that many patients who had had prolonged spasmodic asthma for years did not show emphysema at autopsy. The relatively high incidence of emphysema in asthmatics is most satisfactorily explained by the detrimental effect in such patients of bronchospasm and excess mucus-secretion on the course of attacks of acute bronchiolitis; permanent bronchiolar damage and obliteration are more likely than in similar infections in non-asthmatic individuals.

Interpretations of physiological studies on patients with pulmonary emphysema are often confusing. The changes in emphysema that underlie alterations in the "elasticity" of the lung and in the mechanics of ventilation are particularly debatable. It can be demonstrated easily in emphysematous patients that more force is required, than in normal patients, to move air in and out of the lungs. To state that this is due to loss of "elasticity" of the lungs is an over-simplification.

The forces required to alter the volume of the lungs have been analysed into two components: static and kinetic. Static forces can be measured when air flow in the lungs is assumed to have ceased. By recording the pressure required to maintain a certain lung volume in a patient whose respiration has been temporarily arrested, a pressure-volume curve can be plotted. Decrease of the volume-change normally found with a certain change in pressure is referred to as reduction of "compliance" by Comroe *et alii* (1955), and by other workers as "elastance", "lung tension" or "lung pressure". "Compliance" is essentially a measure of the force overcoming elastic resistance in the lungs. This elastic recoil has several components; von Neergaard and Wirz (1927) adduced evidence that it was largely due to the surface tension of the thin film of fluid lining the alveolar walls. They considered that the elastic tissue of the lung contributed little to elastic retraction—an opinion now supported by most observers. Thus gross fibrosis or carcinomatosis reduces these elastic forces directly, and the measure of this, "compliance", is found to be lower than normal.

Is loss of "compliance" in emphysema due to decrease in these elastic forces? Undoubtedly this plays some part, but Stead *et alii* (1952) found that the "compliance" in emphysematous patients was altered rather than reduced—a similar change being observed in other conditions with an increased functional residual volume. For instance, "compliance" is reduced in patients recovering from bulbar poliomyelitis, in those suffering from an attack of asthma, and in normal subjects under general anæsthesia (Comroe *et alii*, 1955). In all of these states the elastic components are not directly affected; loss of compliance can only be explained by air "trapped" in lung beyond obstructed bronchioles, a phenomenon which would account for both reduction in the efficacy of the forces of retraction and an increase in the functional residual volume.

The next question is whether this loss of compliance accounts entirely for the difficulty of breathing in emphysema. The increased effort required for inspiration and expiration might be thought to be due simply to the circumstance that the resting position of the chest is more expanded than normal. This was shown by Fry *et alii* (1954) to account for only a small part of the resistance; and, since the resistance to ventilation was increased when gases denser than air were breathed, they felt that the major disability in emphysema was due to increased airway resistance—an increase which was present throughout the respiratory cycle, but which was considerably greater during expiration than during inspiration.

Previously, Dayman (1951) had also concluded that, since the same pattern of increased resistance to air-flow could be demonstrated in asthma and in laryngeal obstruction, it was due essentially to airway obstruction. In these conditions, on removal of the obstruction, the changes were completely reversible; in so far as bronchospasm and mucus-obstruction contribute to the respiratory distress in some cases of emphysema, treatment directed towards the relief of these complicating factors often gave some relief. However, no matter what the therapeutic endeavour, considerable air-way obstruction remained demonstrable throughout the respiratory cycle by physiological techniques. Dayman (1951), Fry *et alii* (1954) and Barach (1955) accounted for this residual resistance by the phenomenon of expiratory collapse of bronchioles, but this explanation is not completely adequate, since it does not explain the residual increase of airway resistance in inspiration.

Clearly, the subject requires fuller analysis. In chronic emphysema the basic cause of airway obstruction is bronchiolar obliteration; in the early morphological stages of the disease, in which the lesions are purely focal, disruption of the walls of passages beyond the obstructions and the formation of secondary communications compensate to some extent for the obliteration, and aeration of more distal passages is improved. Quite extensive bronchiolar obliteration can occur and adequate lung function be maintained

at the expense of destruction of some lung tissue at the middle of the lobule. It was observed that the lungs of most men over middle age showed macroscopic focal emphysema (McLean, 1956*a*) ; so that, although Greifenstein *et alii* (1952) selected for lung function studies only subjects over fifty years of age who had no symptoms or signs of cardio-pulmonary disease, many of their patients probably had some degree of pre-clinical emphysema. They found that many of these subjects showed evidence of uneven ventilation of the lungs, but, on the other hand, there was little evidence of decreased lung function other than slight reduction of vital capacity and maximum breathing capacity. These observations are explicable in view of the morphological findings presented here.

With further bronchiolar damage and obliteration, the limit of this " compensatory " mechanism is ultimately reached. At the stage when the whole lobule is involved, further " compensation " of subsequent bronchiolar obliteration by disruption of passages simply decreases the amount of functioning lung tissue. At a roughly corresponding stage of evolution of the disease, expiratory collapse of bronchioles adds to the expiratory resistance. When this phenomenon occurs, it is due, in part, to the air trapped in collaterally ventilated areas compressing the bronchiolar walls. However, as Dayman (1951) pointed out, in advanced emphysema little lung tissue remains to provide traction on the surviving bronchioles during expiration ; so that, at this stage, no matter how free the collateral ventilation that results from disruption of air passages, any expiratory effort will result in collapse of the bronchioles in the affected part.

It is not unreasonable to suggest that in these areas of advanced emphysema the bronchioles remain collapsed throughout expiration. Air enters and leaves these regions only by the tortuous collateral channels created by irregular disruption of the lung tissue, thereby reaching the bronchi through less affected lung. This is in accord with the observations of Fry *et alii* (1954) that led to the conclusion that airway resistance is due largely to air turbulence. Though these authors mention that collateral ventilation may possibly be significant, they do not clearly indicate in what manner it plays a part in emphysema.

The clinical association of chronic bronchitis and emphysema is complex, and the nature of chronic bronchitis is poorly understood. The two are often linked in a clinical syndrome ; and although cases of clinical emphysema with no evidence of chronic bronchitis or asthma are apparently rare in Britain (Christie, 1952) and in America (Kountz and Alexander, 1934), they are not uncommon in Australia (Fitts, 1956). This clinical observation is supported, in this study, by the absence of morphological evidence of chronic bronchitis in patients with early focal lesions. Some lungs with more advanced disease (even a number with gross lesions) did not show inflammatory bronchial changes. It is thus evident that chronic bronchitis does not necessarily accompany emphysema.

Chronic bronchitis is essentially due to low-grade inflammation of the respiratory tract, particularly of the mucus-secreting areas—the nasal mucosa and sinuses, the trachea, bronchi and large bronchioles. It is evidenced histologically by hyperactivity of the mucus-secreting cells and, physiologically, by increased tone of the muscle of the bronchi and bronchioles.

The agents recognized as ætiologically related to the chronic condition are chemical and bacterial. The action of chemical agents is straightforward. They must necessarily be either extremely dilute or of low toxicity ; even brief exposure to the more toxic gases can result in a gross necrotizing bronchiolitis followed, if the patient survives the acute incident, by extensive bronchiolar obliteration (McLean, 1957*a*). The two main agents which are generally accepted as being significant are tobacco smoke and polluted atmospheres.

Although chronic bronchitis is commoner in smokers, and the incidence increases with increasing cigarette consumption (Palmer, 1954 ; Oswald and Medvei, 1955), this does not explain the high incidence of the condition in Britain when compared with Scandinavia (Goodman *et alii*, 1953) or Australia. The only recognizable variant in these circumstances is the degree of atmospheric pollution. In Britain, the mortality due to bronchitis in males has been correlated with the consumption of domestic coal per acre (Daly, 1954) and with the atmospheric concentration of sulphur dioxide (Pemberton and Goldberg, 1954).

Moderate exposure to these agents would not be expected to produce significant direct bronchiolar damage. Their main effect is to stimulate mucus-production ; this hyper-secretion in the young adult ordinarily does not overload the ciliary mechanism of disposal of secretions, but when it does, coughing effectively ejects accumulated mucus. However, following acute viral infections, in young adults exposed to these chemical agents, the phase of secondary

bacterial infection often persists for days or even weeks after the viral damage is over. The factors underlying this persistence of bacterial infection are significant. In a study of acute bronchiolitis (McLean, 1956*b*) it was concluded that since bacteria constantly enter the bronchial tree in health, establishment of bacterial infection is necessarily due to failure of host homœostatic mechanisms. The inflammatory response results from absorption of the products of bacteria and phagocytes. The degree of inflammation depends on the concentration of organisms; this in turn depends largely on the duration of retention of infected material at the site. The maximal inflammatory response to infections was found in the small bronchioles, since, in these passages, aspirated infected plugs could remain static for relatively long periods. Thus, even though this may not be apparent clinically, bacterial inflammation is bronchiolar rather than bronchial.

In the healthy adult the commonest cause of failure of homœostasis is viral infection; although some secondary bacterial infection almost inevitably occurs after the viral phase of inflammation, the exudate and excess mucus-secretion (due to secondary bacterial infection) ordinarily is readily expelled, and bacterial infection subsides. If chemical irritants add to the hypersecretion of mucus, bacterial damage may persist somewhat longer. Since these chemical agents increase the bronchiolar inflammation and the damage resulting from such infections, they therefore form one group of the many accessory factors of ætiological significance in the genesis of emphysema.

However, chemical agents are clearly not the whole basis of chronic bronchitis. Oswald (1954) found that the usual age of onset of the chronic productive cough was thirty to fifty years, yet these patients had usually been subject to atmospheric pollution all their life, and those who smoked had presumably done so for years; clearly another factor is operative. In many such cases mucus aspirated bronchoscopically is sterile (Allison *et alii*, 1943; Benstead, 1950); so that the suggestion that the added factor is bacterial infection is inadequate (and in any case is begging the question).

The added factor is, simply, emphysema. That this is so is indicated by the morphological evidence that emphysema precedes clinical or morphological evidence of bronchitis. An accurate concept of the place of emphysema in chronic bronchitis has been provided recently by physiological studies on cough velocities. Although many authors have stated that the efficacy of cough in emphysema is reduced (Dayman, 1951; Fry *et alii*, 1954; Comroe *et alii*, 1955), work such as that of Barach *et alii* (1953) and Barach (1955) has placed the subject on a sounder (physical) footing. Measuring air velocities as volume of air expelled from the lungs per unit time, they found that the maximal velocity of cough in a normal person was approximately 10,000 cubic centimetres per second, and that velocities over 6000 cubic centimetres per second were adequate to deal with retained secretions; below this figure clearing of secretions was inadequate. None of their patients with clinical emphysema could exceed this figure, and many produced maximal cough velocities under 3000 cubic centimetres per second. Insufficient work has been reported to indicate at what stage of the disease "cough failure" occurs, and it would be of value to investigate patients with "chronic bronchitis" by these techniques before they develop obvious clinical emphysema.

The stage of emphysema at which cough becomes inadequate to deal with accumulating secretions depends on the degree of mucus-production. A chronic cough therefore develops relatively early (morphologically) in the disease in patients exposed to considerable concentrations of chemical irritants, but in those not exposed to inhaled chemical agents cough may never become troublesome, particularly if the ciliary mechanism is not overloaded.

When cough becomes ineffective (either locally as in bronchiectasis, or generally as in advanced emphysema), retention of secretions in the bronchial tree necessarily follows. Bacteria entering the lower part of the respiratory tract are retained longer, and, at this stage, prolonged bacterial inflammation may result. The sputum becomes purulent, initially only after episodes of acute inflammation (most of which are presumably viral in origin); with increasing emphysema and decreasing efficacy of coughing after such an episode, in the absence of effective therapy or change in the environment, the sputum may eventually remain purulent indefinitely. That this bacterial inflammation is primarily bronchiolar is evident in the morphological study of chronic bronchitis presented by Reid (1954).

The bacterial flora of these infections has not yet been completely elucidated. Most frankly purulent infections were due to *Hæmophilus influenzæ* or pneumococci, and a few were due to staphylococci (Mulder *et alii*, 1952; May,

1953*a*, 1953*b*, 1954). Such organisms were uncommonly present in mucoid sputum, in which there were, microscopically, many leucocytes and bacteria. In mucoid sputa, non-specific organisms such as *Streptococcus viridans* or *Neisseria catarrhalis* were cultured (May, 1953*a*), and the possibility cannot be denied that such organisms, when present in sufficient concentration, may induce some inflammatory response.

Thus chronic bronchitis is usually the result of prolonged exposure to inhaled chemical irritants (the action of which may precede the development of morphological emphysema) together with chronic bacterial infection, which ensues only at a stage of "cough failure"—usually induced by a combination of hypersecretion of mucus and a considerable degree of morphological emphysema. The significance attached to chronic bronchitis as a cause of emphysema in Britain is clearly an indication of the degree of pollution of the urban atmosphere in that country.

From the morphological viewpoint non-specific bronchiolitis is the basic lesion of emphysema. Although diffuse bronchiolar disease may be due occasionally to such conditions as tuberculosis, silicosis or damage by toxic gases, by far the most important condition is acute bronchiolitis due primarily to the common viral diseases with secondary infection by the bacterial flora of the nasopharynx. The damage done by these common infections depends on their number, duration and severity. Any accessory factor that results in increased number, or prolongation of the bacterial phase, of such infections will increase the rate of evolution of the disease.

Even without the operation of accessory factors, some diffuse bronchiolar damage (and emphysema) can result from repeated uncomplicated respiratory tract infections (McLean, 1957*a*, 1957*b*). The moderate degree of emphysema found at autopsy in such patients dying at an advanced age is often referred to as "senile" emphysema—a term which has little significance, and which is no less vague than the corresponding clinical description of "senile" emphysema of Kountz and Alexander (1934).

In patients with clinical emphysema under the age of fifty years accessory factors can almost always be recognized. A few patients are encountered in whom such factors are not operative, and in these disability can be traced from a specific incident (or incidents) in which extensive bronchiolar damage and obliteration would be expected, such as prolonged "atypical pneumonia", staphylococcal pneumonia or exposure to toxic gases (McLean, 1957*a*).

The predominance of the clinical disease in males cannot be adequately explained. Smoking is probably a significant factor, since Oswald and Medvei (1955) showed that, among smokers, there was no difference in the incidence of chronic cough between sexes, suggesting that the sex difference is simply accounted for by the observation that men smoke more than women. Other possible factors, such as the relatively higher force of coughing in males or sex-linked hereditary disposition to excessively severe respiratory infections, have received little support or investigation.

ACKNOWLEDGEMENTS

This work was carried out during the tenure of a grant from the National Health and Medical Research Council.

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A COMPARISON OF THE EARLY METABOLIC EFFECTS OF TRI-IODOTHYRONINE AND HYDROCORTISONE IN MAN[1]

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SUMMARY

The early metabolic effects of intravenous triiodothyronine and hydrocortisone have been studied in five healthy male volunteers over a ten-hour period.

Triiodothyronine (0·5 and 1·0 milligramme) produced a rise in metabolic rate with a fall in non-protein respiratory quotient in three hours. This was associated with an increase in serum and urinary inorganic phosphate content but no increase in total nitrogen or electrolyte excretion.

Hydrocortisone (50 milligrammes) produced a rise in potassium excretion after two hours, followed by a retention of sodium. There was an initial retention followed by an increase in urinary excretion of phosphate associated with a rise in serum inorganic phosphate content. There was also a rise in serum cholesterol content.

These changes have been compared with those associated with stress in man. Though neither hormone reproduced the full stress pattern, each reproduces some of the changes, and hence they may both be involved in their mechanism.

THE purpose of this study of the early metabolic effects of tri-iodothyronine and hydrocortisone was to shed light on the mechanisms involved in the pattern of metabolic changes associated with stress in man. The main features of this pattern are well known—an increase in metabolic rate, increased protein and fat catabolism, diminished carbohydrate tolerance and certain changes in electrolyte metabolism (Selye, 1950). This pattern of changes is found with infection (Grossman *et alii*, 1945; Burns *et alii*, 1953), post-operatively (Moore and Ball, 1952) and following fractures (Cuthbertson, 1932). Recently a similar pattern of changes under conditions of emotional disturbance produced by stressful life situations has been described (Hetzel *et alii*, 1956*a*, 1956*b*).

The development within recent years of accurate methods for the estimation of the level of adrenal cortical hormone in body fluids (blood and urine) has established the occurrence of definite increases both post-operatively (Moore, 1954) and with emotional disturbance (Hetzel *et alii*, 1955; Bliss *et alii*, 1956; Board *et alii*, 1956).

These increases in hormone levels appear to be brought about through activation of the adrenal by adrenocorticotrophic hormone (ACTH), the pituitary being first stimulated from the hypothalamus. As the metabolic effects of ACTH can be reproduced by hydrocortisone (Fourman *et alii*, 1950; Thorn *et alii*, 1953), it is now generally accepted that hydrocortisone is the main secretory product of the adrenal when it is stimulated by ACTH. Hence this agent has been used (now that it is available and suitable for intravenous injection), and the effects have been compared with those of stress in order to determine how much of this pattern it will reproduce.

The question of the possible role of the thyroid gland in the metabolic changes during stress has been largely overlooked until recently. This is because thyroxin has been regarded as the only thyroid hormone. The well-known latent period between the administration and the metabolic effect of this substance (about two days) (Asper *et alii*, 1953; Rawson *et alii*, 1953) seemed to preclude the participation of the thyroid in what after all were rapidly developing changes occurring within hours of exposure to stimuli.

The isolation of the more rapidly acting hormone tri-iodothyronine by Gross and Pitt Rivers in 1952 has produced a modification in our concepts of thyroid physiology. The precise physiological significance of tri-iodothyronine,

[1] Received on February 11, 1957. This work was supported by the Michell Foundation for Medical Research, University of Adelaide.

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however, is still undecided. These present studies were carried out in an effort to clarify this important point.

METHODS

Observations were made on five healthy volunteer male medical students on each of three occasions one week apart after fasting overnight.

An infusion of normal saline at the rate of two millilitres per minute was given over a period of four hours each day, beginning between 8 a.m. and 9 a.m. It was given alone as a placebo, and to this was added 50 milligrammes of hydrocortisone[1] on one occasion and 0·5 milligramme (or 1·0 milligramme) tri-iodothyronine[2] on another occasion to make up three different treatments. Each treatment was given on each of the three days in random fashion.

Samples of blood and urine were collected on arrival at the laboratory at approximately 8 a.m. Observations were made on: (*a*) The serum content of inorganic phosphate, urea, lipids, sodium and potassium at two-hourly intervals for ten hours. (*b*) The urine flow and urea, total nitrogen, inorganic phosphate, sodium, potassium and creatinine excretion at two-hourly intervals. (*c*) The gaseous exchange—oxygen consumption, carbon dioxide elimination and respiratory quotient (R.Q.) at two-hourly intervals one, three, five and seven hours from the beginning of the infusion. This was done using the Hartmann-Braun basal metabolic rate indicator. In each case observations were made at one-minute intervals for ten minutes and the mean values taken. Determination of the total nitrogen excretion permitted calculation of the non-protein respiratory quotient (N.P.R.Q.).

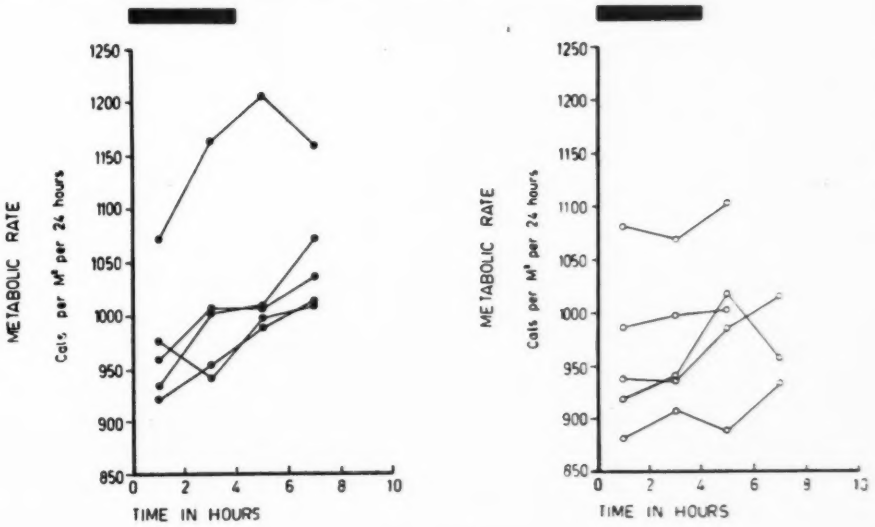

FIGURE I

Metabolic rate following administration of tri-iodothyronine (filled-in circles) and placebo (open circles)

Techniques and methods used were: EEL flame photometer for the electrolyte determinations, the method of Taussky and Shorr (1953) for inorganic phosphate, micro-Kjeldahl with Nesslerization for total nitrogen, the urease method for serum urea content and the method of Bonsnes and Taussky (1945) for creatinine. The serum cholesterol levels were determined by the method of Zlatkis *et alii* (1953); the serum phospholipids by the method of Brown (1954) and the serum total esterified fatty acids by the method of Stern and Shapiro (1953) as modified by Day *et alii* (1956).

RESULTS

(1) *Changes in Metabolic Rate*

The effect of tri-iodothyronine in the five subjects is shown in Figure I.

[1] The generous gift of Upjohn Co., Kalamazoo, Michigan.

[2] The generous gift of Glaxo Laboratories Ltd., Greenford, Middlesex.

TABLE I

Effect of Tri-iodothyronine on Metabolic Rate (Calories per Square Metre per Twenty-four Hours)

Subject	Treatment[1]	Dose (Milligrammes)	S.A.	Time in Hours			
				1	3	5	7
A	T_3	1·0	1·98	959	1008	1006	1037
	P			986	999	1004	—
B	T_3	0·5	1·94	921	954	989	1014
	P			882	908	888	934
C	T_3	0·5	1·86	934	1004	1010	1072
	P			938	936	986	1017
D	T_3	1·0	2·07	1071	1163	1205	1159
	P			1081	1069	1103	—
F	T_3	0·5	1·79	977	942	998	1009
	P			918	941	1020	957
Mean				972	1014	1042	1058
				961	971	1000	969
P				N.S.	<0·1	<0·2	<0·05

[1] T_3 = tri-iodothyronine. P = placebo.

It will be seen that consistent increases were obtained over the seven-hour period. The changes observed with 0·5 milligramme were not strikingly different from those with 1·0 milligramme. This has been confirmed by subsequent additional observations.

TABLE II

Effect of Tri-iodothyronine (T_3), Hydrocortisone (F) and Placebo (P) on Serum Inorganic Phosphorus Content. (Milligrammes per 100 Millilitres)

Subject	Treatment	Time in Hours					
		0	2	4	6	8	10
A	T_3	3·4	3·3	3·8	4·3	4·5	4·8
	P	2·8	3·2	3·6	3·4	3·3	3·2
	F	2·2	2·4	2·6	3·0	2·5	4·2
B	T_3	3·6	3·0	2·8	3·0	4·5	5·1
	P	4·7	4·4	4·6	4·0	3·8	4·0
	F	3·6	3·7	3·3	3·6	4·7	5·0
C	T_3	4·5	3·8	4·4	4·8	4·6	4·6
	P	3·2	3·8	3·6	3·9	3·7	3·9
	F	3·9	3·2	3·1	4·9	4·3	5·4
D	T_3	3·7	3·5	3·7	4·0	5·0	4·7
	P	3·6	2·7	2·4	2·5	3·1	3·2
	F	3·2	2·7	3·4	4·1	5·4	4·9
F	T_3	4·8	3·7	3·1	4·9	4·7	5·3
	P	5·0	4·1	4·0	4·5	4·4	4·4
	F	4·4	3·9	3·0	4·3	4·5	5·6
Mean	T_3	4·0	3·5	3·6	4·2	4·7	4·9
	P	3·9	3·6	3·6	3·7	3·7	3·7
	F	3·5	3·2	3·1	4·0	4·3	5·0
P[1]	T_3	N.S.	N.S.	N.S.	N.S.	<0·05	<0·001
	F	N.S.	N.S.	N.S.	N.S.	N.S.	<0·001

[1] Significance of difference between tri-iodothyronine and placebo or between hydrocortisone and placebo.

TABLE III

Effect of Tri-iodothyronine (T_3), Hydrocortisone (F) and Placebo (P) on Urinary Phosphorus Excretion (Milligrammes per Minute)

Subject	Treatment	Time in Hours					
		0	0–2	2–4	4–6	6–8	8–10
A	T_3	0·49	0·22	0·50	0·56	0·92	1·15
	P	0·52	0·37	0·53	0·50	0·54	0·69
	F	0·48	0·33	0·19	0·05	0·30	0·68
B	T_3	—	0·14	0·21	0·29	0·66	1·48
	P	0·21	0·05	0·25	0·53	0·37	0·05
	F	0·50	0·11	0·14	0·06	0·26	0·75
C	T_3	0·86	1·20	0·98	1·09	1·40	1·08
	P	0·43	0·30	0·35	0·45	0·37	0·32
	F	0·78	0·42	0·22	0·21	0·63	1·15
D	T_3	0·67	0·79	0·35	0·77	1·42	1·23
	P	1·46	0·39	0·32	1·59	0·52	0·39
	F	0·38	0·26	0·08	0·44	0·87	1·47
F	T_3	0·75	0·45	0·56	0·89	0·88	0·76
	P	1·45	0·62	0·62	0·58	0·54	0·46
	F	1·27	0·59	0·28	0·20	0·67	1·49
Mean	T_3	0·55	0·56	0·52	0·72	1·05	1·14
	P	0·81	0·34	0·41	0·73	0·46	0·38
	F	0·68	0·34	0·18	0·19	0·54	1·11
P[1]	T_3	N.S.	N.S.	N.S.	N.S.	<0·01	<0·05
	F	N.S.	N.S.	<0·05	<0·05	N.S.	<0·05

[1] As for Table II.

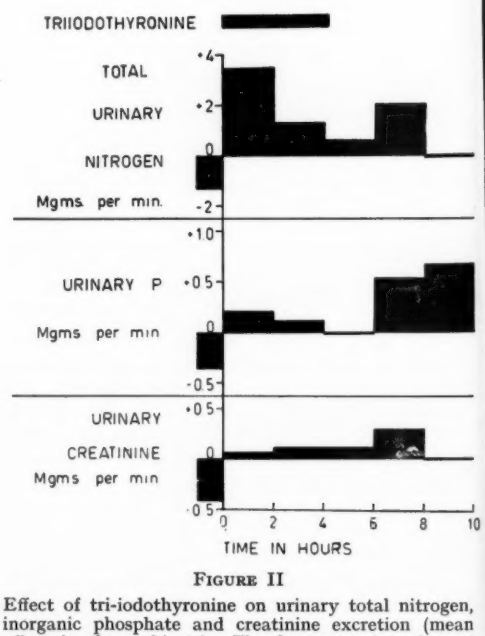

FIGURE II

Effect of tri-iodothyronine on urinary total nitrogen, inorganic phosphate and creatinine excretion (mean effect for five subjects). The figures express the net effect, *i.e.* the difference between the corresponding figures for tri-iodothyronine and placebo. If above the horizontal line, the value for T_3 is greater than for placebo, if below the line, the value is less than for placebo

The effect of the placebo on the five subjects is also shown in Figure I. It will be seen that there was a tendency for the metabolic rate to rise after three hours. This is probably attributable to the greater mobility of the subjects after removal of the intravenous infusion. Detailed figures are given in Table I. Analysis of these results reveals a significant rise ($P<0\cdot05$) present in seven hours. The increase after three hours does not reach this level of significance ($P<0\cdot10$). However, pooling of the results with those obtained from two more subjects subsequently did reveal a significant increase ($P<0\cdot05$) at three hours.

Comment.—It is concluded that tri-iodothyronine caused an increase in metabolic rate within three to seven hours. Owing to limitations of time, no determinations of metabolic rate were made following administration of hydrocortisone.

(2) *Effects on Protein Metabolism*

No significant increase in serum or urinary urea or total nitrogen content was observed with either agent under these conditions. A significant increase in serum inorganic phosphorus content was observed following tri-iodothyronine at eight hours (Table II). This was associated with a corresponding increase in inorganic phosphate excretion (Table III and Figure II). This increase appeared to be in proportion to the increase in metabolic rate, for the increase in serum phosphate correlated with the increase in metabolic rate in subjects showing both "good" and "poor" response to the administration of tri-iodothyronine (Figure III).

Hydrocortisone produced an initial retention of phosphate (two to six hours) followed by a subsequent phosphate diuresis (Figure IV, Table III).

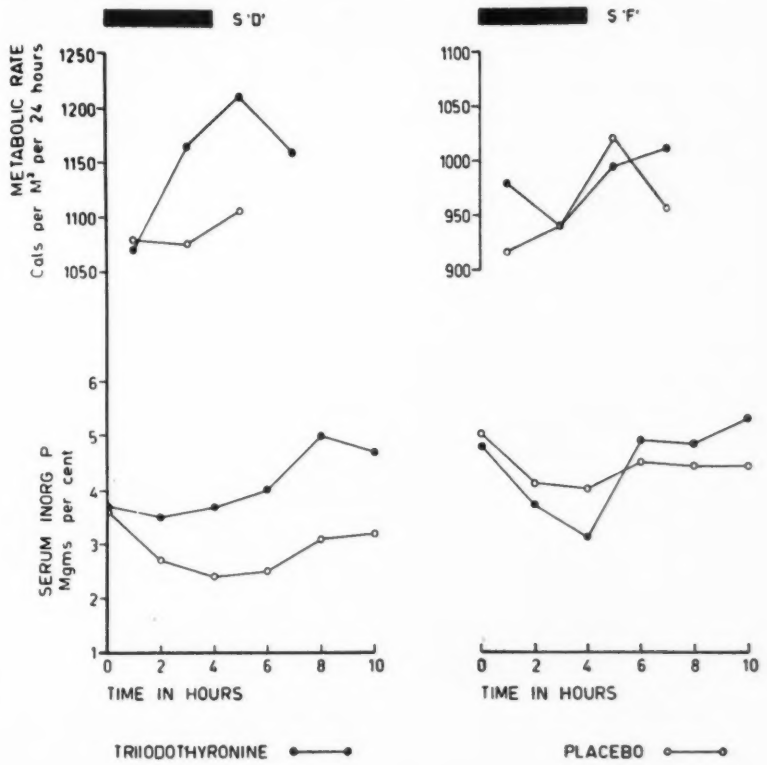

FIGURE III

Effect of tri-iodothyronine on metabolic rate and serum inorganic phosphate content in a "good" reactor and a "poor" reactor

This latter increase was associated with a rise in the serum phosphate content, which was significant at ten hours (Table II). These changes in serum and urinary phosphate levels would appear to be related to a simultaneous increase in potassium excretion and will be discussed below.

No significant increase in creatinine excretion occurred with either agent.

Comment.—Changes in phosphorus excretion in excess of nitrogen were observed with both tri-iodothyronine and hydrocortisone.

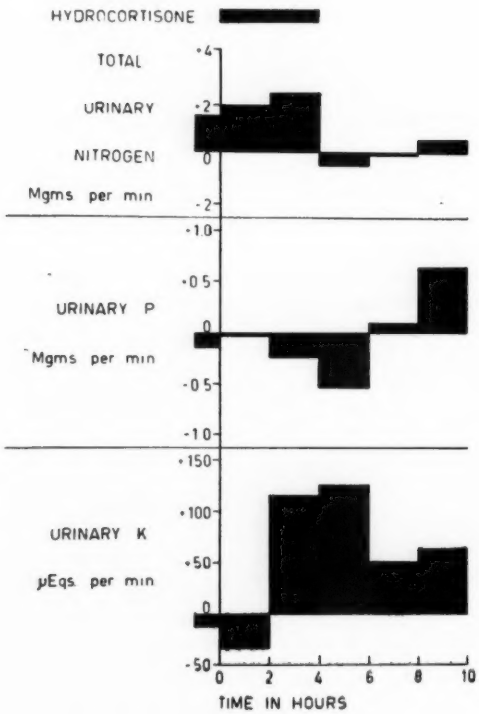

FIGURE IV

Effect of hydrocortisone on urinary total nitrogen, inorganic phosphate and potassium excretion (mean effect for five subjects). (See legend to Figure II)

(3) *Effects on Fat Metabolism*

Calculations of the N.P.R.Q. following administration of tri-iodothyronine revealed a significant fall at three hours ($P<0{\cdot}05$), but this was not maintained (Table IV). A rise in serum cholesterol content followed the administration of hydrocortisone (Figure V). A transient fall was evident with tri-iodothyronine, but this did not reach statistical significance (Table V).

No effect was observed on either the serum fatty acid or phospholipid levels with either agent within ten hours.

TABLE IV

Effect of Tri-iodothyronine (T_3) and Placebo (P) on Non-protein Respiratory Quotient (N.P.R.Q.)

Subject	Treatment	Dose (Milligrammes)	Time in Hours 1	3	5	7
A	T_3	1·0	0·82	0·79	0·80	0·79
	P		0·81	0·81	0·75	—
B	T_3	0·5	0·75	0·75	0·76	0·73
	P		0·80	0·79	0·78	0·76
C	T_3	0·5	0·81	0·77	0·74	0·79
	P		0·82	0·83	0·78	0·77
D	T_3	1·0	0·72	0·72	0·71	0·77
	P		0·75	0·74	0·74	—
F	T_3	0·5	0·79	0·82	0·78	0·77
	P		0·79	0·83	0·80	0·79
Mean	T_3		0·78	0·77	0·76	0·76
	P		0·79	0·80	0·77	0·77
P			<0·2	<0·05	N.S.	N.S.

Observations five days after administration of one milligramme of tri-iodothyronine to another subject revealed a definite fall in R.Q.

TABLE V

Effect of Tri-iodothyronine (T_3), Hydrocortisone (F) and Placebo (P) on Serum Cholesterol Content. (Milligrammes per 100 Millilitres)[1]

Subject	Treatment	Time in Hours 0	2	4	6	8	10
A	T_3	185	178	177	172	152	158
	P	187	198	187	191	193	201
	F	198	190	205	222	196	195
B	T_3	200	192	211	204	200	210
	P	185	174	176	184	199	201
	F	188	182	173	208	196	203
C	T_3	203	213	202	213	213	228
	P	204	216	190	195	200	218
	F	190	195	211	212	225	211
D	T_3	212	228	202	205	222	258
	P	204	219	215	215	215	215
	F	213	220	230	235	230	248
F	T_3	215	220	218	230	228	236
	P	192	185	181	194	210	201
	F	233	226	248	250	257	243
Mean	T_3	203	206	202	205	203	218
	P	194	198	190	196	203	207
	F	204	203	213	225	221	220

[1] Analysis of orthogonal polynomial coefficients shows: (i) Comparing F and P—divergence at four to eight hours is significant. $0{\cdot}01<P<0{\cdot}02$ (quartic component analysis). (ii) Comparing T_3 and P—divergence at four to eight hours does not reach significance. $0{\cdot}10<P<0{\cdot}20$ (cubic component analysis).

serum cholesterol and phospholipid levels. No change in the level of serum fatty acids occurred (Figure VI).

Comment.—These results suggest that the increase in metabolic rate following the administration of tri-iodothyronine is due partly to an increased combustion of fat.

(4) *Effects on Electrolyte Metabolism*

With tri-iodothyronine, no significant effect was observed on the level of serum sodium or potassium, nor was there any alteration in the rate of excretion of these elements in the urine (Tables VI and VII). The rate of urine flow remained unchanged except for a transient increase at the six to eight hour period (Figure VII).

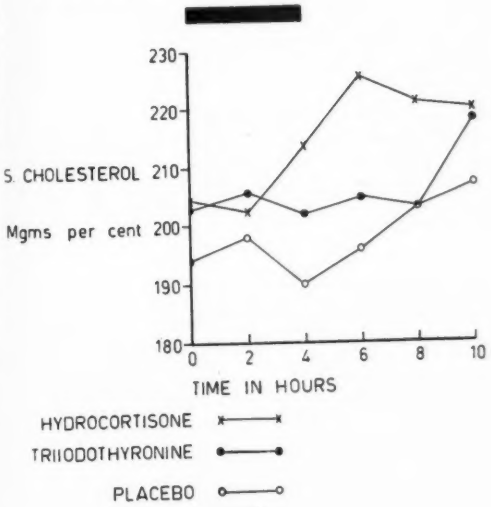

FIGURE V

Effect of hydrocortisone and tri-iodothyronine on serum cholesterol content (means of five subjects)

With hydrocortisone an early and marked increase in potassium excretion occurred (Table VII, Figure VIII). However, this increase, as noted previously, was not accompanied by any increase in either nitrogen or phosphate excretion. In actual fact an initial significant retention of phosphate occurred, although this was followed later by an increased rate of excretion in parallel with the rise in potassium excretion (Figure IV). An increase in serum potassium content was also observed by two hours, but did not reach statistical significance. Following the potassium diuresis there was a striking sodium retention (Figure VIII, Table VI). There was also a transient increase in urine flow followed by a retention accompanying the sodium retention (Figure VIII).

DISCUSSION

It will be apparent that each agent has reproduced some of the metabolic changes associated with stress. An increase in metabolic rate was produced by tri-iodothyronine within three to seven hours. Increases of metabolic rate have not been observed following the administration of cortisone to man in the

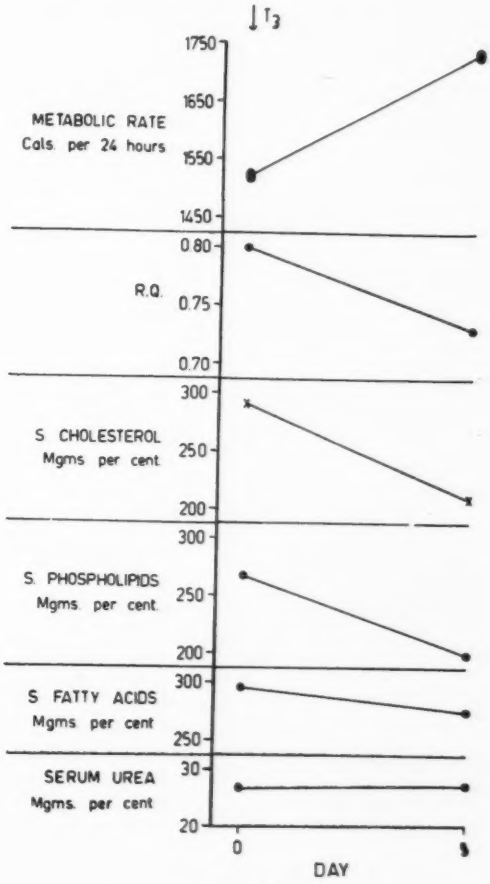

FIGURE VI

Effect of tri-iodothyronine (one milligramme) on fat metabolism in one subject after five days

euthyroid state, although a slight increase may occur when cortisone is given to hypothyroid subjects (Hills *et alii*, 1950).

It is of interest that no significant increase in nitrogen excretion occurred within ten hours of the administration of tri-iodothyronine. An increase might have been expected in association with the rise in metabolic rate. This would

suggest that the increased catabolism does not involve protein at this stage. A subsequent increase in nitrogen excretion does occur, however, within twenty-four hours (Rawson *et alii*, 1953). No increase in serum non-protein nitrogen or urea content has been observed following administration of tri-iodothyronine either early or late (Asper *et alii*, 1953). As expected, no change in nitrogen excretion occurred as the result of hydrocortisone administration (Thorn *et alii*, 1953).

TABLE VI

Effect of Tri-iodothyronine (T_3), Hydrocortisone (F) and Placebo (P) on Urinary Sodium Excretion. (Micro-equivalents per Minute)

Subject	Treatment	Time in Hours					
		0	0–2	2–4	4–6	6–8	8–10
A	T_3	152	183	206	275	217	162
	P	140	160	172	108	163	190
	F	173	210	222	147	76	65
B	T_3	144	104	186	174	90	81
	P	113	240	342	581	250	161
	F	147	76	176	95	25	74
C	T_3	86	232	209	191	239	122
	P	220	256	340	229	188	125
	F	131	237	271	163	34	72
D	T_3	189	222	272	380	335	177
	P	207	236	300	234	178	71
	F	167	458	447	209	51	73
F	T_3	274	440	494	570	266	200
	P	384	263	315	282	282	201
	F	353	537	454	145	80	81
Mean	T_3	169	236	273	318	229	148
	P	213	231	294	287	212	149
	F	194	304	314	152	53	73
P[1]	T_3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	F	N.S.	N.S.	N.S.	N.S.	<0·01	<0·05

[1] As for Table II.

The increase in both serum and urinary inorganic phosphate content following administration of tri-iodothyronine without a comparable increase in nitrogen excretion suggests that a breakdown of high energy phosphate is occurring. It is probable that both phosphocreatine and adenosine triphosphate are involved, and there may be in addition an uncoupling of oxidative phosphorylation (Lardy and Maley, 1954). Similar changes have been noted over longer periods in hypothyroid subjects given tri-iodothyronine and thyroxine (Rawson *et alii*, 1953; Asper *et alii*, 1953). It would appear, therefore, that these compounds are an important source of the extra energy mobilized by tri-iodothyronine. However, during stress, increases in phosphate excretion are usually in proportion to increases in nitrogen excretion (Cuthbertson, 1932).

The associated significant fall in N.P.R.Q. with tri-iodothyronine after three hours indicates that some shift from carbohydrate to fat metabolism had occurred by this stage. There was also a slight fall in the level of serum cholesterol, but no significant change occurred in the levels of phospholipid or fatty acids. Observations in one instance five days after tri-iodothyronine administration revealed a fall in both the serum cholesterol and phospholipid levels. Similar changes after a prolonged interval have been noted following the administration of tri-iodothyronine to patients with myxœdema (Rawson *et alii*, 1953; Asper *et alii*, 1953) and also following the administration of thyroid to euthyroid subjects (Strisower *et alii*, 1954). These observations suggest that the increased metabolic rate which occurs both early and late is in part a result of increased fat catabolism. Similar changes occur during stress in man (Cuthbertson, 1932; Groen *et alii*, 1951; Hetzel *et alii*, 1956*a*).

A potassium diuresis was the predominant feature of the effect of hydrocortisone. This increase in potassium excretion was associated with an initial retention of phosphate, but was later followed by an increase in phosphate output and a retention of sodium. The changes observed in the distribution of these electrolytes have been attributed to the mobilization of

TABLE VII

Effect of Tri-iodothyronine (T_3), Hydrocortisone (F) and Placebo (P) on Urinary Potassium Excretion. (Micro-equivalents per Minute)

Subject	Treatment	Time in Hours					
		0	0–2	2–4	4–6	6–8	8–10
A	T_3	77	133	98	81	51	57
	P	105	108	69	35	33	45
	F	95	106	162	157	118	90
B	T_3	—	113	65	45	19	34
	P	107	130	106	111	37	30
	F	248	45	225	182	63	57
C	T_3	33	115	105	60	37	25
	P	97	143	125	100	61	42
	F	108	126	215	219	75	73
D	T_3	96	190	129	87	87	47
	P	262	286	198	81	33	43
	F	75	177	342	306	130	85
F	T_3	81	127	112	85	33	35
	P	84	46	66	51	45	57
	F	74	121	227	154	83	76
Mean	T_3	58	135	102	71	45	40
	P	131	143	113	75	42	43
	F	120	115	234	204	93	76
P[1]	T_3	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
	F	N.S.	N.S.	<0·001	<0·01	<0·01	<0·001

[1] As for Table II.

intracellular potassium and its replacement by sodium. Phosphate is retained at first in an endeavour to offset the increase in cation. This retention continues until increased amounts of phosphate become available from the cells

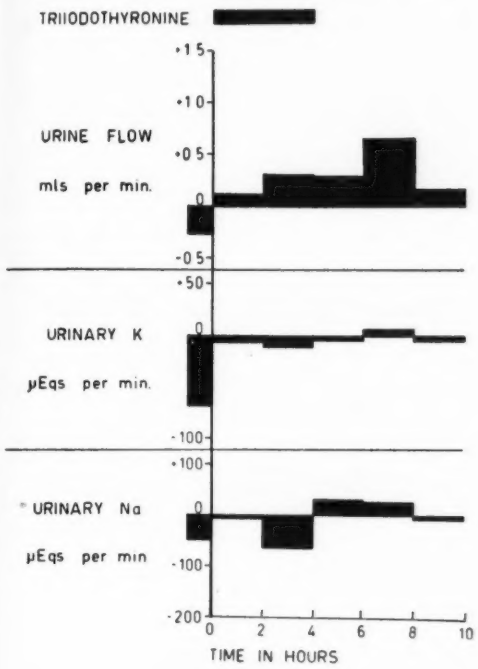

FIGURE VII

Effect of tri-iodothyronine on urine flow, sodium and potassium excretion (mean effect for five subjects). (See legend to Figure II)

(Fourman *et alii*, 1950). An increase in the serum potassium level has been shown to occur following the intravenous administration of a dose of 100 milligrammes of hydrocortisone (Knight *et alii*, 1955). In this investigation with a dose of 50 milligrammes a rise in serum potassium occurred, but did not reach statistical significance. Similar changes occur during stress in man (Moore, 1954).

A rapid and marked increase in serum cholesterol content was the other striking feature of hydrocortisone administration. The reason for this change remains obscure—it may be related to its known effect on mobilizing depot fat (Levin and Farber, 1952).

It is concluded, therefore, that these two hormones together reproduce many of the features of the metabolic response associated with stress in man. The role of the adrenal requires no emphasis, but the possibility that the thyroid plays an important role has not been fully explored.

Evidence of increased thyroid activity under conditions of exposure to low temperature is available both in animals (Bondy and Hagewood, 1952) and in man (Ingbar *et alii*, 1953). On the basis of animal studies it has also been suggested that the thyroid plays an important role in the production of the profound metabolic changes following burns (Sellers *et alii*, 1950; Gribble and Peters, 1951; Wase *et alii*, 1953), but investigation in man (Cope *et alii*, 1953) has failed to disclose evidence of thyroid overactivity in such circumstances. A possible role of the thyroid in the post-operative response in man has also recently been suggested

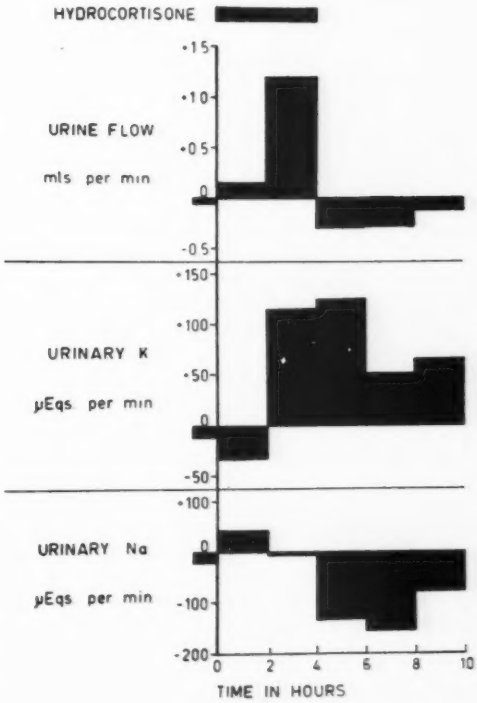

FIGURE VIII

Effect of hydrocortisone on urine flow, sodium and potassium excretion (mean effect for five subjects). (See legend to Figure II)

(Goldenberg *et alii*, 1955). Finally, the role of the thyroid in the metabolic response associated with emotionally stressful life situations has been examined and suggestive evidence of its importance obtained (Hetzel *et alii*, 1956*a*, 1956*b*).

In the past, little interest has been shown in this likely role of the thyroid gland in the production of some of the metabolic changes associated with stress. This has been due to the widely held concept of the long "latent period" of the thyroid hormone. The discovery and isolation of tri-iodothyronine in conjunction with the present data on the early metabolic effects of this agent indicate that this is not necessarily the case. Moreover, this would appear to be confirmed by the observation of a similar rapid increase in metabolic rate following thyrotropic hormone administration (Hetzel *et alii*, to be published). The apparent specificity of the increase indicates that such rapid rises may occur under physiological conditions. Clinicians have long been familiar with the rapidly developing thyroid crisis in thyrotoxicosis. This also may have some physiological basis in a rapidly acting thyroid hormone.

It is of interest that there was no significant increase in total nitrogen or electrolyte excretion following the administration of tri-iodothryonine for such changes have been previously reported following thyrotropic hormone (Hetzel and Plescia, 1955). The probable specificity of these changes following the administration of thyrotropic hormone supports the possibility previously suggested by Hetzel and Plescia (1955) that this hormone stimulates the secretion of an agent distinct from tri-iodothyronine. This will be discussed further elsewhere.

ACKNOWLEDGEMENTS

We wish to thank Professor H. N. Robson for help with necessary facilities; Dr. H. R. Gilmore, Dr. A. Day, Dr. C. Schwartz[1] and Mrs. G. Wilkinson for the lipid determinations; Dr. A. T. James and Mr. G. Wilkinson, of Division of Mathematical Statistics, C.S.I.R.O., for the statistical analysis; and Miss B. Leach and the nursing staff of the Royal Adelaide Hospital for general assistance.

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[1] Working with the support of the National Health and Medical Research Council of Australia.

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OBSERVATIONS ON THE NATURE OF HORMONE–INDUCED EOSINOPENIA[1]

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SUMMARY

The possible causes for the disappearance of eosinophils from the peripheral blood following the administration of certain adrenal steroid hormones are reviewed.

A series of experiments was performed in which the number of circulating eosinophils was determined at regular intervals following the intravenous administration of either ACTH or hydrocortisone.

The results of these experiments show that under these circumstances the rate of disappearance of eosinophils is approximately constant. In no instance was the rate of disappearance described by an exponential function.

It is claimed that this constant rate of disappearance implies an inhibition of the production or delivery of eosinophils by the bone marrow to the peripheral blood ; and that those cells in the circulation fulfil their normal physiological life span.

The points in time at which the eosinopenic reaction is maximal have been determined. It is claimed that these represent estimates of the life span of eosinophil leucocytes. The range of such estimates is from 165 to 377 minutes.

Other methods used to estimate the survival of leucocytes, in general, and eosinophils, in particular, are discussed in the light of these present observations.

SINCE "the coarse granular corpuscles" were first described among the formed elements of the blood more than one hundred years ago (Jones, 1846), these cells, later designated as eosinophil leucocytes by Ehrlich (1879), have been a source of fascination to many investigators. Despite this intense interest in their structure and function, their role in the economy of the body has remained enigmatic.

During these years there has been much speculation about the structure of the eosinophil. Their coarse granules, possessing as they do so strong an affinity for acid dyes, have been alleged to contain glycogen (Habershon, 1906), protein (Rous, 1908) and phospholipid (Sheehan, 1939). It would now seem generally agreed that these granules consist of a phospholipid surface surrounding a central protein core (Bloom and Wislocki, 1950). It has been claimed that these cells are responsible for the production of Charcot-Leyden crystals (Ayres, 1949).

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To the eosinophil has been ascribed the function of histamine release (Code, 1937 ; Vaughn, 1953), of the transport of antigens and antibodies (Godlowski, 1948) and even of the secretion of red blood cells (Duran-Jorda, 1947). The clinical conditions associated with an increase in the number of circulating eosinophils have been appreciated for the past six decades. Ringoen (1938) has pointed out that an increase in circulating eosinophils is a common and definite feature of allergic and hypersensitive states. Although it was suggested that this eosinophilia occurred only as the result of the union of antigen and antibody and was not seen in the non-sentitized individual, Vaughn (1952) has recently shown that blood and tissue eosinophilia in guinea-pigs may be produced by a single injection of the soluble fraction of ascaris extract. This implies that the eosinophil response is an intrinsic property of the extract and not dependent upon an antigen-antibody reaction. Many attempts have been made to discover a common factor involved in the production of eosinophilia (Spiers, 1955), but to date none has been found.

Until recently those conditions associated with a reduction in the number of circulating eosinophils have not been associated with much experimental or clinical study. Zappert

(1893) observed that in severe infections eosinophils disappeared from the circulating blood at the height of the infection and returned to normal during the period of convalescence, an observation which has been repeatedly confirmed during the past fifty years.

Further attention was focused on this reaction by the experiments of Dalton and Selye (1939), who described the characteristic triad of peripheral blood effects: lymphopenia, eosinopenia and neutrophilia in animals subjected to stress by formaldehyde injections or severe exercise. These effects have more recently been incorporated into Selye's concept of "the general adaptation syndrome", the eosinopenic effect being part of the "alarm reaction" (Selye, 1949). The significance of this observation was not fully appreciated until the introduction of corticotrophin (ACTH) into clinical medicine. Hills *et alii* (1948) showed that when ACTH was administered to individuals with normal adrenocortical function there was an abrupt and conspicuous decrease in the numbers of circulating eosinophils. This observation, which has been confirmed in humans and animals, forms the basis of one of the clinical tests of adrenocortical function (Thorn *et alii*, 1948). Since then a number of experiments have been performed to elucidate the nature of this hormone-induced eosinopenia.

It has been shown that the group of hormones responsible for this phenomenon are those adrenal steroids with an oxygen in the 11-position (Halberg, 1952). Of these steroids, 17-hydroxycorticosterone (hydrocortisone) and 11 - dehydro - 17 - hydroxycorticosterone (cortisone) are the most potent. By contrast, mineralo-corticoids are without eosinopenic properties (Thorn *et alii*, 1955).

Although there is general agreement as to which hormones possess eosinopenic properties, no convincing evidence has been adduced as to how this phenomenon is mediated. Several hypotheses have been advanced in explanation, but each lacks convincing experimental support. In general terms eosinopenia may result from failure of production or delivery of eosinophils by the bone marrow, excessive destruction of eosinophils in the peripheral blood, or redistribution of eosinophils within internal organs or systems.

The presently available evidence does not support the view that hormone-induced eosinopenia results from a failure of production or delivery of eosinophils by the bone marrow. Studies on the bone marrow of humans before and during treatment with adrenal steroids have generally failed to detect any consistent or conspicuous changes in the eosinophil patterns of the marrow at the time when peripheral blood eosinopenia is marked (Finch *et alii*, 1951; Quittner *et alii*, 1951; Root *et alii*, 1953). Durgin and Meyer (1951) have, however, observed changes in the eosinophil pattern following the subsidence of the acute eosinopenic reaction; these changes consisted of an increase in the percentages of young eosinophilic cells and were presumed to be a compensatory reaction to replace eosinophil leucocytes in the peripheral blood. Uhrbrand (1954) also noted a rise in bone-marrow eosinophils after the administration of ACTH. More recently, Fruhman and Gordon (1955) have made quantitative studies on the bone marrow of rats. They have shown that treatment with cortisone, in doses sufficient to produce substantial eosinopenia in the peripheral blood, results in a significant depression of the numbers of eosinophils in the bone marrow. They are unable to draw firm conclusions as to whether this represents an increased release of eosinophils to the circulation or an inhibition of their formation. Hayes and Baker (quoted by Gordon, 1955) have reported that chronic treatment of intact rats with adrenocortical extract results in a significant reduction of the percentages of eosinophils in the bone marrow.

The direct effect of adrenal steroids on eosinophils has been investigated. Godlowski (1952) and Muehrcke *et alii* (1952) reported that there was destruction of eosinophils *in vivo* and *in vitro* by cortisone. Padawer and Gordon (1952*a*, 1952*b*) reported that during hormone-induced eosinopenia there was an increased eosinophil destruction. These experiments have not been substantiated. With regard to degenerating eosinophils in the peritoneal cavity, under the conditions of their experiment similar changes were also observed by Durham (1897), who described chromatolysis, karyorrhexis and cytoplasmic fragmentation of eosinophils and other nucleated cells. These are, in fact, the changes that may be seen in any inflammatory exudate. Several workers (Baldridge *et alii*, 1951; Cape, 1952; Hudson, 1954*a*) have failed to confirm the initial reports by Godlowski and Muehrcke of the direct effect of cortisone on eosinophils. At present it would seem that there is little, if any, convincing evidence that hormone-induced eosinopenia results from the direct destruction of eosinophils.

Another mechanism that has been invoked as a cause of hormone-induced eosinopenia is the redistribution of eosinophils from the circulation into other organs or systems. Spain and Thalhimer (1951) have suggested

that the eosinopenic reaction originates in part, at least, from the migration of eosinophils from the circulation into organs such as the spleen. Their evidence, an increased number of eosinophils in the spleens of mice under the influence of cortisone, appears most inadequate. The eosinopenic response in splenectomized humans does not differ from that of normal humans (Essellier *et alii*, 1954; Hudson, unpublished observations). In dogs, Solomon and Humphreys (1951) were unable to demonstrate any significant splenic uptake during ACTH-mediated eosinopenia.

Essellier *et alii* (1954) claim that the cause of hormone-induced eosinopenia is the sequestration of eosinophils in the reticulo-endothelial system under the influence of adrenal steroids. This claim is based upon the inhibition of cortisone-induced eosinopenia in guinea-pigs by preliminary "blockade" of the reticulo-endothelial system by India ink or Trypan blue dye. Essellier admits that this inhibition can be "broken through" if the dose of hormone is increased. Hudson (1954*b*) has shown that similar doses of India ink and Trypan blue failed to prevent cortisone-induced eosinopenia in guinea-pigs. Neither series of experiments is sufficiently critical to uphold or refute the hypothesis that hormone-induced eosinopenia results from increased sequestration of eosinophils within the reticulo-endothelial system. As far as we are aware there is no other evidence to support this hypothesis.

This paper is concerned with the study of this problem from another aspect. From the time the eosinopenic response was first described, it was generally recognized that maximal eosinopenia occurred approximately four hours after the application of the stimulus. This time relationship is the basis of the four-hour Thorn test for adrenocortical insufficiency (Thorn *et alii*, 1948). The theoretical considerations which led to the following study are those which have been elaborated for the study of the survival of erythrocytes. It has been known for some years that when "tagged" normal erythrocytes are transfused into a normal recipient, there is a gradual disappearance of these cells from the circulation (Ashby, 1919). Under the conditions outlined, all the cells have disappeared from the circulation after approximately one hundred and twenty days. A linear function results if the numbers of transfused cells remaining in the circulation are plotted against time. This linear function implies that the cells are lost from the circulation on the basis of their age; and that each cell has approximately the same life span, which is determined by congenital or intrinsic factors, its disappearance or death being attributed to senescence (Eadie and Brown, 1953). When abnormal conditions prevail, erythrocytes disappear in a random fashion, the removal of cells from the circulation occurring without regard to age. Under such conditions, the relationship between the number of surviving cells and time follows an exponential function.

This concept may be applied to the study of hormone-induced eosinopenia. After the application of a potent eosinopenic stimulus, a curve may be determined by plotting the mean counts observed during eosinophil disappearance against the time after the stimulus. On a-priori grounds, this curve may take a linear, exponential or some other form. For this series of observations the two extreme forms of curve will be considered: the broken linear and the simple exponential. If the curve constructed by plotting mean cell concentration against time is a broken linear one, it follows that the eosinopenic response is due to a sudden and substantial, but not necessarily complete cessation of production or delivery of eosinophils by the bone marrow, with senescence accounting for the subsequent disappearance of these cells from the blood. If, on the other hand, this curve has a simple exponential form, one might infer that the cells were randomly destroyed, almost without regard to age—such destruction as might occur if the eosinopenia were due to intravascular lysis or redistribution of the cells into one or more internal organs or systems. Should it be shown that the disappearance of eosinophils under the influence of adrenal steroids traced a broken linear curve, then, as a corollary, the point in time at which maximal eosinopenia was observed would provide an estimate of the intravascular life span of these cells.

The results of this series of experiments represent the study of 16 patients. They indicate that after the administration of corticotrophin or hydrocortisone the disappearance of eosinophils traces a linear curve, and that the time when this response is maximal lies between 165 and 370 minutes. It is suggested, therefore, that eosinopenic response to adrenal steroids results from an inhibition of the production or delivery of eosinophils by the bone marrow; and that the intravascular life span of these cells lies between 165 and 370 minutes.

MATERIALS AND METHODS

The enumeration of eosinophils was performed as has already been described (Hudson, 1954*a*) using a modified Randolph stain and Fuchs-

Rosenthal counting chambers (depth, 0·2 millimetre).

At each time eosinophils were counted, two pipettes were filled with capillary blood from a finger and diluted with staining fluid. After a suitable interval one side of each of two Fuchs-Rosenthal chambers was filled from each pipette. Thus, at any given time the total number of cells contained in 12·8 cubic millimetres of diluted blood was counted. In every experiment the enumeration of eosinophils was performed by the same observer and completed within the day of the experiment.

Sixteen patients were studied on nineteen separate occasions. The disorders which necessitated their admission to hospital are contained in Table IV. In every instance the hormones were administered intravenously. Some patients were given ACTH, 50 international units in 500 millilitres of dextrose solution over a period of six to eight hours, and others hydrocortisone, 100 milligrammes in 250 millilitres of dextrose solution over a period of thirty to sixty minutes. The patients were fasting throughout the experiment. Blood for cell counts was collected in all but one patient at forty-minute intervals after the start of the infusion, for periods of at least 300 minutes and up to 400 minutes. In this one patient the counts were made at sixty-minute intervals.

TABLE I
Patient No. 16[1]

Time (Minutes)	Number of Cells Counted				Totals (12·8 Cubic Millimetres)
0	87	69	85	87	328
40	56	64	58	62	240
80	55	62	43	46	206
120	36	40	47	42	165
160	22	19	21	22	84
200	12	10	8	9	39
240	6	8	3	6	23
280	4	4	4	6	18
320	4	1	2	7	14
360	8	5	5	2	20

[1] 100 milligrammes of hydrocortisone given intravenously on July 20, 1955.

RESULTS AND STATISTICAL TREATMENT

It is felt that it would be inappropriate to reproduce in detail the individual counts on all the patients studied. Table I shows in detail the numbers of eosinophils counted at each time from patient No. 16. These counts are a representative sample of the remainder of the patients studied.

Two hypotheses were considered in the statistical investigation of the observations. Both hypotheses postulated that at a time, t, after the initial count, the observed number of eosinophils was a random variable following the Poisson distribution with mean (and variance) equal to μ, μ_t varying with t in a fashion specified by the particular hypothesis.

The use of the Poisson distribution in this context requires some explanation. In a previous investigation, Hudson and Binet (1956) found empirically that the negative binomial distribution fitted the counts better than the Poisson. In particular, they postulated that the probability of observing a count r, when the mean is μ_t and the variance is $\mu_t+\frac{\mu_t^2}{\theta}$ is:

$$\binom{\theta+r-1}{\theta}\left(\frac{\mu_t}{\mu_t+\theta}\right)^r\left(\frac{\theta}{\mu_t+\theta}\right)^\theta,$$

where θ is a constant. The data yielded the estimate 296·8 of θ. Using the negative binomial distribution and putting $\theta=296{\cdot}8$, estimates of the relevant parameters can be found, with a method similar to that described below for the Poisson distribution. Calculations along these lines were performed for two patients and yielded estimates which were the same (to the number of significant figures used) as those obtained under the Poisson assumption. Having regard to the large amount of computation required when the negative binomial distribution was employed, the simpler Poisson hypothesis was used in all subsequent calculations.

The first hypothesis postulated that the mean decreased exponentially with time (the time being measured from the instant when the eosinophil count was highest), namely:

$$\mu_t=\mu_0 e^{-Kt}$$

where μ_0 and K are positive constants.

The second hypothesis postulated that the mean decreased linearly with time over the range $(0, \tau)$ whereafter it remained constant, that is:

$$\mu_t=\begin{cases}\alpha+\beta t, & \ldots\ldots\ 0\leqslant t\leqslant\tau,\\ \alpha+\beta\tau, & \ldots\ldots\ t\geqslant\tau,\end{cases}$$

where α and τ are positive constants, and β is a negative constant of absolute magnitude

less than α/τ (the time being measured as in the first hypothesis).

The value of the time τ at which μ_t became steady will be referred to subsequently as the "cut-off point", and $\alpha+\beta\tau$, the value of μ_t at this point, as the threshold value.

Both hypotheses were fitted to the data from one group of patients (Nos. 1 to 5). The results of this fitting indicated that for the remainder of the observations the second hypothesis was the more suitable one to examine.

For the first hypothesis, the constants were fitted by weighted least squares, the computations being performed iteratively. This yielded estimates m, k for the parameters μ_0 and K, and it was then possible to make χ^2 tests on the agreement of the data with results predicted from the hypothesis. Two weighted sums of

TABLE II

Patient	Deviations from Hypothesis		Deviations from Poisson Distribution		F Test $F=\frac{(\chi^2/\text{d.f.})_H}{(\chi^2/\text{d.f.})_P}$
	χ^2_H	d.f.$_H$	χ^2_P	d.f.$_P$	
1	61·8†	8	35·3	36	(7·9,† 8, 36 d.f.)
2	92·6†	7	83·4†	31	(4·9,† 7, 31 d.f.)
3	19·4*	8	32·5	34	(2·5,* 8, 34 d.f.)
4	11·6*	5	19·8	28	(3·3,* 5, 28 d.f.)
5	21·6†	7	45·3*	28	(2·5,* 7, 37 d.f.)

d.f. = degrees of freedom.
* Significance at the 5% level.
† Significance at the 1% level.
All other values do not differ significantly from predicted value.

squares were calculated for each set of observations, the first being a sum of squares of deviations of the sample means from the predicted curve, and the second a sum of squares showing the residual variability of the individual observations about their sample means. The first of these two statistics is a measure of the effectiveness of the hypothesis put up for the behaviour of μ_t, while the second is a measure of the adequacy of the Poisson hypothesis as representing the distribution of the observations at a fixed point in time. Since both these sums approximately follow a χ^2 distribution, theoretical values are available against which each of them can be tested. Where the result of the test on the Poisson hypothesis shows a significant deviation, a valid F test can still be performed by which the deviation of the sample means from the predicted curve is tested against the internal variability of the sample. The results of these calculations are shown in Table II.

It may be noted that in no patient did the exponential curve provide a satisfactory model for predicting the behaviour of the sample means, and that in two patients (Nos. 2 and 5) the residual variation about the sample means exceeded that which would be expected from the Poisson distribution. In these, the sum of squares arising from the deviations between the sample means and their predicted values was still significantly greater than can be accounted for by the internal variability of the data.

TABLE III

Patient	Deviations from Hypothesis.		Deviations from Poisson Distribution		F Test
	χ^2_H	d.f.$_H$	χ^2_P	d.f.$_P$	
1	10·4	5	20·3	28	
2	49·5†	8	83·8†	37	2·7,* 8, 37 d.f.
3	2·6	7	33·4	34	
4	4·9	5	21·4	28	
5	6·9	7	47·1	40	
6	4·0	4	31·8* (low)	49	1·5, 4, 49 d.f.
7	4·4	6	28·1	27	
8	9·0	5	38·0*	24	1·1, 5, 24 d.f.
9	8·6	5	28·1	24	
10	7·6	5	31·8	24	
11	3·5	5	20·5	24	
12	22·0†	6	44·0*	27	2·3, 6, 27 d.f.
13	4·4	6	16·2	27	
14	2·9	6	30·9	27	
15A 9/8/55	3·6	6	21·5	27	
15B 12/8/55	11·8	7	30·4	30	
16A 20/7/55	7·0	7	22·4	30	
16B 21/7/55	23·1†	7	25·9	30	(3·8,† 7, 30 d.f.)
16C 19/8/55	4·3	7	24·9	30	

d.f. = degrees of freedom.
* Significance at the 5% level.
† Significance at the 1% level.
All other values do not differ significantly from predicted value.

The F values in parentheses (patients Nos. 1, 3 and 4) show that this latter conclusion is also true for these patients.

The second hypothesis was then examined. Since the parameter τ defines a range of values of t, it was not possible to use conventional maximum likelihood methods for its estimation. However, once τ had been placed between two given consecutive values of t, maximum likelihood estimates a, b of α and β could be found. This difficulty was overcome by examining the graphs of the sample means and

selecting two or three possible locations for τ. The estimates α and β were then calculated for each of these locations, and from these estimates a second estimate of τ was made for each initial location. The threshold value of y was also calculated. This was found to be the arithmetic mean of all the sample points lying beyond τ, a result which is intuitively reasonable. The likelihood associated with each set of estimates for α, β and τ was then calculated, the choice being governed by that set of estimates which gave the largest likelihood. It was now possible to calculate the same χ^2's as had been estimated for the former hypothesis. These are shown in Table III in which the same abbreviations are used as in Table II.

On examining the second hypothesis it will be noted that only four of the patients yield a χ^2 which is significantly different from that predicted by the Poisson hypothesis. The value obtained in Patient No. 6 is significantly low, a result which admits no simple explanation and is possibly due to chance alone. For only three patients is the sum of squares due to sample means significantly high, and in one of these (Patient No. 12) the F test yields a non-significant result. The results obtained in Patient No. 2 are anomalous since neither hypothesis is satisfactory at any stage. Apart from this patient the only serious departure from the second hypothesis is shown by the results for Patient No. 16 on July 21, 1955. On this day the magnitudes of the eosinophil counts were considerably lower than those obtained on the other two days when the patient was given hydrocortisone, and it is possible that the count on this day was still being influenced by the drug from the previous day.

The foregoing evidence suggests that the second hypothesis is the more acceptable one. The estimates of τ for the 19 series of observations are given in Table IV, together with the hormone administered and the values of the initial and lowest eosinophil counts (cells per cubic millimetre), each value being the sum of the counts from four chambers. In two patients, given corticotrophin, there was some delay between the time of administration of the hormone and the time at which the eosinophil count commenced to fall. In these instances the maximal count has been entered in lieu of the initial count.

TABLE IV

Patient	Disease	Drug Given	Initial Count (Cells per Cubic Millimetre)	Maximal Eosinopenia (Cells per Cubic Millimetre)	Estimate of τ (Minutes)
1	Anxiety State	ACTH	127	39	165
2	Scleroderma	ACTH	545	20	307
3	Scleroderma	ACTH	1040	230	286
4	Rheumatoid arthritis	ACTH	211	16	173
5	Arteriosclerosis	ACTH	316	30	183
6	*Anorexia nervosa*	ACTH	91	9	223
7	Postural hypotension	ACTH	178	22	377
8	Hypothyroidism	ACTH	734	72	348
9	Arteriosclerosis	Hydrocortisone	194	8	219
10	Arteriosclerosis	Hydrocortisone	725	34	252
11	Post-partum pituitary necrosis	Hydrocortisone	175	30	235
12	Addison's disease	Hydrocortisone	518	20	206
13	*Anorexia nervosa*	Hydrocortisone	128	11	252
14	Nervous dyspepsia	ACTH	70	11	252
15A	Addison's disease	Hydrocortisone	532	42	205
15B	Addison's disease	Hydrocortisone	532	17	217
16A	Addison's disease	Hydrocortisone	514	30	214
16B	Addison's disease	Hydrocortisone	292	5	292
16C	Addison's disease	Hydrocortisone	402	44	184

DISCUSSION

These observations and their statistical analysis indicate that under the influence of adrenal steroids the disappearance of eosinophils from peripheral blood is better approximated by a broken linear curve generated by a constant rate of disappearance due to senescence than by a simple exponential curve given by random disappearance. We feel that such a conclusion admits but one biological interpretation: following the application of the eosinopenic stimulus there is a temporary, abrupt and nearly complete cessation of the supply of eosinophils to the peripheral blood by the bone marrow. Those cells in the circulation fulfil their normal intravascular life span. Their physiological aging process is reflected by their approximately constant rate of disappearance.

Both hypotheses regarding the behaviour of μ_t assume that the active steroid does not have a differential effect on cells of different ages. However, if it is postulated that a differential aging effect is present, either alone or associated with the two hypotheses proposed earlier, it can be shown that the expected mean number of cells present at time t after the application of the stimulus can be represented by a curvilinear function of t. A significant departure from linearity by the sample means would therefore suggest that there is a genuine differential aging effect (assuming that the exponential hypothesis had been examined and refuted). However, Table III shows that no such departure occurs, except in Patient No. 2, and in the second set of observations of Patient No. 16. As there is reason to believe that both these sets of observations represent departures from the normal pattern of the development of eosinopenia, the hypothesis of the differential effect of the active agent on the eosinophils due to their age was not considered further.

If all the circulating eosinophils were affected equally by the active agent, then their disappearance would take place randomly and on the basis of cell concentration. Such a reaction must be represented by a decay curve with exponential character. The character of the disappearance of cells redistributed into internal organs or systems would also be random, and therefore the curve would be exponential in form. It is clear that in none of the patients studied do the disappearance curves even approximate to exponential decay.

It follows that if the rate of disappearance of eosinophils is approximately uniform, as has been shown by the analyses of these experiments, then the point in time when eosinopenia is maximal provides an estimate of the intravascular life span of these cells. In this series of experiments the estimates of this time varied between 165 and 377 minutes. Although this is a wide range of times, examination of the estimates shows them to be approximately log-normally distributed with a mean time of 250 minutes.

Over the past thirty years many estimates of the intravascular life span of leucocytes have been made by a number of different investigators. These estimates have varied widely in accordance with the methods used for the determination of cell survival. An early observation by Minot and Richards (1925) was that leucocytes from a patient with lymphatic leuchæmia, when transfused into a patient with lymphosarcoma, had disappeared from the circulation at the end of two hours. Weiskotten (1930) examined rabbit bone marrow after benzol administration and concluded that the average duration of life of neutrophils in rabbits' blood was between three and four days. Yoffey (1935) measured the rate of flow and lymphocyte concentration in major lymphatic channels in dogs and estimated the life span of lymphocytes to be of the order of eleven to twelve hours. Osgood (1937) has made observations on the survival of leucocytes in tissue culture; he claims that in this medium eosinophils survive for as long as twelve days. While these observations are no doubt valid for this artificial medium, it is not justifiable to transpose them to the survival of eosinophils within the circulation, where they encounter considerably greater stresses and strains than in the sheltered existence of tissue culture. Lawrence *et alii* (1945) deduced that the survival time of leucocytes was of the order of sixteen hours. This estimate was based upon the rate of disappearance of leucocytes administered to leucopenic cats by cross circulation experiments. A similar experiment was performed by van Dyke and Huff (1951), who used parabiotic rats, one parabiont of which was rendered leucopenic by irradiation. Their estimate of cell survival was one hundred and seventy minutes for monocytes and twenty-three minutes for neutrophil leucocytes. Other investigators have attempted to label leucocytes, vitally, with dyestuffs: Farr (1946) used acriflavine hydrochloride and observed a small number remaining in the circulation up to seventy-two hours, while White (1954) studied the intravascular life span of transfused leucocytes tagged with atebrine. This latter author found that the tagged cells remained in

the circulation for periods of up to ninety minutes.

More recently leucocytes have been labelled *in vitro* and *in vivo* by radioactive isotopes. Kline and Clifton (1952) incorporated P^{32} into nuclear desoxypentose nucleic acid. Similar studies were made by Ottesen (1954). The former authors deduced a life span of 12·8 days from the time the labelled leucocytes are in the circulation in large numbers. Ottesen observed a similar lag period between the administration of P^{32} and the appearance of radioactivity in the peripheral blood. He noted that a peak was reached by the sixth day, which was then followed by a sudden decline in radioactivity. He interprets these results as a slow maturation of cells in the bone marrow, the cells taking five or six days to mature ; and the subsequent rapid decline is the result of the short intravascular life span—less than one day. Weisberger and Levine (1954) have studied the fate of leucocytes into which they had incorporated S^{35}-labelled cystine, and have deduced an approximate white cell survival time of thirteen days. McCall *et alii* (1955) have made a similar estimate by their observations on the fate of leucocytes tagged with Cr^{51}.

It is apparent that, as demonstrated by a wide variety of methods, there exist substantial differences of opinion about the intravascular survival time of leucocytes. A fundamental objection to those methods which have used homologous transfusions or cross-circulation procedures with labelled leucocytes is the probability that the manipulations involved in tagging or transfusing render the cells more vulnerable to the normal processes of destruction. Moreover, it has been shown by transfusing labelled leucocytes (Weisberger *et alii*, 1951 ; Bierman, 1955) that the lungs play a major role in the prompt removal of the transfused leucocytes from the circulation with the liver and spleen playing smaller parts.

An additional objection to many of the methods used for the estimation of the intravascular life span of leucocytes is that none of the methods, particularly those using radioactive isotopes, is able to single out and estimate the survival time of any one type of leucocyte. It would seem quite probable that lymphocytes survive longer than neutrophils, which may, in turn, be longer-lived than eosinophils. To some extent survival must be related to the function of each particular series of cells, and, in terms of metabolic activity, our ideas as to individual white cell function are at the best speculative. In our present state of knowledge we feel that these previous estimates of leucocyte longevity in no way vitiate the results reported in this paper.

Recently Essellier, Jeanneret and Morandi (1954) claim to have estimated the survival time of eosinophils by an indirect method, and one in which manipulative or in-vitro procedures are not utilized. They had observed that following the eosinopenia resulting from large doses of ACTH, there is commonly a period in which the level of circulating eosinophils exceeds the pre-treatment level. They state that after a relatively fixed time there is a sudden fall in the concentration of circulating eosinophils. They have observed this to occur approximately six days after the eosinopenic episode, and claim that it represents the death of those eosinophils produced by the marrow to offset the initial eosinopenia. Although these observations may be valid, we feel that the conclusions drawn from them are highly speculative and permit no critical evaluation. Implicit in their use of the term " regeneration eosinophilia " (following ACTH) is that marrow depression occurs at the time of hormone-induced eosinopenia, and lends further support to this present series of observations—that the eosinopenia resulting from the administration of adrenal steroids results from a failure of production or delivery of eosinophils by the bone marrow to the peripheral blood.

ACKNOWLEDGEMENTS

The authors wish to thank Professor M. H. Belz and Dr. F. E. Binet, of the Department of Statistics, University of Melbourne, and Dr. G. S. Watson, Department of Statistics, Australian National University, for their helpful advice and discussion in planning these experiments and evaluating the results obtained, and Miss Gwenda Jacobs for her assistance in the computations. The helpful advice and criticism of Professor R. D. Wright and Dr. T. E. Lowe are gratefully acknowledged.

The hydrocortisone used in these studies was made available through the generosity of the Upjohn Company, Kalamazoo, Michigan, United States of America.

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GLYCOGEN STORAGE DISEASE WITH UNUSUAL RENAL LESIONS

REPORT OF A CASE WITH AUTOPSY AND ENZYME STUDIES[1]

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SUMMARY

A case is reported of Von Gierke's disease diagnosed at autopsy in a thirty-one-year-old man.

Glycogen isolated from the patient's liver constituted 4·7% of the weight of the organ, and was normal in all respects including ultracentrifugation.

Glucose-6-phosphatase activity was much diminished in the patient's liver, as compared with a normal control. Lactic dehydrogenase activity was equal in both samples.

Renal lesions included an aneurysm of the renal artery, distension of the convoluted tubules with glycogen and nodular glomerular lesions resembling those seen in *diabetes mellitus*.

THE appearance of the liver in hepato-renal glycogen storage disease has been repeatedly described since Von Gierke's (1929) original autopsy reports. The diffuse enlargement of the organ, the distension and vacuolation of the hepatic cells, the increase in portal connective tissue and the varying amounts of fatty change are well known and will not be further discussed here. (See Bonnin and Bonnin, 1951.)

Renal changes, on the other hand, and especially their clinical manifestations, are rarely mentioned. Thus Richtsmeier and Allen (1953), describing a case of Von Gierke's disease with albuminuria and microscopic hæmaturia, were unable to find any association reported in the literature between glycogen storage disease and urinary abnormalities. One of Schulman and Saturen's (1954) three cases showed heavy albuminuria, "rare" red cells, white cells and granular casts in the urine. The patient, who was studied during infancy, survived, and further renal studies were not performed. Most significant of all is the case of Mason and Anderson (1955). Their patient, a girl aged six months with characteristic clinical, microscopic and biochemical findings of glycogen storage disease, showed mild albuminuria. The urine contained 10 to 15 leucocytes and occasional erythrocytes per high power field. At autopsy, ten years later, the kidneys showed not only the expected glycogen deposition in the convoluted tubules, but also "wire-looping" of the glomeruli, an increase in interstitial connective tissue and calcium deposition in some of the tubules.

In this paper a further case of Von Gierke's disease is reported. Autopsy showed three kinds of renal lesions, two of which have, apparently, not so far been described in association with this condition.

CLINICAL RECORD

S.A.S. (R.A.H. 7998/57), a thirty-one-year-old labourer, was admitted to hospital on November 28, 1956, because of coma of twenty hours' duration.

The patient had been born of unrelated parents after a normal pregnancy. Shortly after birth, the mother overheard one of the nurses say the child was jaundiced. She herself, however, never saw any evidence of this. He ran on hands and feet "like an animal" and did not talk until he was aged two years. At that time he was brought to the Alfred Hospital, Prahran, Victoria, because of general backwardness and abdominal swelling. On examination an enlarged liver was discovered. Laparotomy was performed because of suspected primary hepatoma. At operation, the liver was found to be evenly enlarged and very fatty. Biopsy showed the liver cells to be swollen with large vacuoles "consistent with fatty degeneration". The fact that the material was fat was confirmed by special fat stains.

After the operation the patient made a good recovery and developed more or less normally, but was always small for his age. He had a ravenous appetite and was continually thirsty, often drinking four glasses of water at one sitting. He passed large quantities of urine (up to a litre and a half during the night) and his bowels acted three to four times daily. Fatty foods made him vomit and caused an increase in the number of his stools. Other complaints included a chronic aural discharge and shortsightedness, but he was able to attend school, reached grade V, and learnt to play the violin.

When aged eighteen, he suddenly grew up to normal adult size. Sexual development appeared normal, but he was never interested in girls. In fact he developed psychotic tendencies, such as shaving his body hair. He worked as a labourer in several jobs, and was, for a time, employed as garbage collector by the local council.

At the age of twenty-seven, he developed epileptic fits. His head would turn to the right, and this was

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followed by a generalized convulsion. Examination showed hypertension (blood pressure 200/120 mms. Hg) and hepatomegaly. Investigations performed at this stage included a complete blood picture, blood urea nitrogen estimation, liver function tests and intravenous pyelography. All were within normal limits.

During the last few months before admission to hospital, he had been losing weight. His epileptic fits, however, appeared well-controlled with various anticonvulsant drugs (one fit every six to eight weeks), and the family were not unduly concerned when he was found unconscious on the day prior to his admission to hospital. He had been in coma twenty hours before being brought into hospital.

There was no distinct episode diagnosed as kidney disease in the past history. On direct questioning the mother admitted that the patient had been dragging his right leg, but she was not sure how long this sign had been present.

The family history was significant. Four paternal uncles and aunts had died in infancy of unknown causes. The patient's father had always drunk " gallons " of water as well as alcoholic beverages. He had also been psychotic for many years, and had been arrested by the police on several occasions because of indecent exposure. During a recent physical examination, Dr. Noel Chenhall, of Box Hill, Victoria, had found no evidence of hepatomegaly, hypertension or urinary abnormalities. The patient's mother, his only sister and the sister's daughter were in good health. His only brother had died at the age of four years of an illness diagnosed as diphtheria.

Examination showed an unconscious man. The temperature was 103·4° F., the pulse rate 120 per minute, the respiration rate 30 per minute, and the blood pressure 100/65 mms. Hg. He appeared dehydrated. The left pupil was larger than the right, both pupils reacting sluggishly to light. The liver was diffusely enlarged, the right lobe being palpable six fingers' breadth below the right costal margin. A distinct notch could be felt between the right and left hepatic lobes. The right tendon jerks were increased, and there was a right-sided Babinski reflex.

The urine had a specific gravity of 1·008, and examination showed a trace of acetone, no sugar and a " two plus " reaction for albumin. The blood urea nitrogen content was 31 milligrammes per 100 millilitres, the serum sodium content 150 milliequivalents per litre, serum potassium content 6·1 milliequivalents per litre, and the serum chloride content 98 milliequivalents per litre. Lumbar puncture showed grossly blood-stained cerebro-spinal fluid under extremely low pressure. Three hours after admission to hospital the patient suddenly went into a tonic spasm, became cyanosed, and died.

POST-MORTEM FINDINGS

Macroscopic Examination

Autopsy (No. 10657) was performed twenty hours after the patient's death. External appearances showed a well nourished man looking older than his stated age. The facial appearance was not abnormal. The chest, abdomen and pubic area had been recently shaved, and there was a right paramedian, upper abdominal scar. The right thigh muscles showed considerable wasting compared with the left.

The heart was much enlarged (weight 460 grammes), most of this enlargement being due to left ventricular hypertrophy. The myocardium was otherwise normal to naked eye inspection.

The lungs showed posterior basal congestion, but were otherwise normal.

The liver was very much enlarged (weight 4820 grammes) and of a greyish-pink colour and cut with a slightly gritty sensation. The cut surface showed a slightly granular appearance with some fine yellow mottling. This appearance was uniform throughout both lobes with the exception of a darker nodule, one centimetre in diameter, in the left lobe.

The kidneys were both enlarged, weighing 250 and 230 grammes. Their cut surface was a greyish-pink, of a somewhat darker hue than that seen in the liver.

FIGURE I

Structures at the hilum of the right kidney. One branch of the renal artery is given off proximal to the aneurysm and one emerges from the aneurysm itself. The second aneurysm lies in the bifurcation of the artery into its main upper and lower trunks

There was some fine yellow stippling of cortex and medulla. The cortico-medullary ratio was normal, and there was no evidence of any abnormality in the renal pelves or ureters. Just distal to the first branching of the right renal artery one of its primary trunks showed an aneurysmal dilatation three-quarters of a centimetre in diameter. A second aneurysm half a centimetre in diameter was present a few millimetres distal to the first (Figure I). There was no evidence of occlusion or narrowing of the arterial lumen at either aneurysm or anywhere else along the course of the renal artery and its extra-renal branches.

The spleen was slightly enlarged (230 grammes), but otherwise normal.

The brain showed an area of hæmorrhage one and a half centimetres in diameter in the pons. This had ruptured into the subarachnoid space on the left and, medially, into the fourth ventricle. There was some wasting of the left pyramid. No aneurysm or angioma could be demonstrated to account for the pontine hæmorrhage, and no lesion was found above the level of the pons.

The pituitary was normal.

FIGURE II

" Plant-like " hepatic cells, considerably larger than normal. Hæmatoxylin and eosin. (×330)

Microscopic Appearances

The liver cells were swollen and " plant-like " with no nuclear displacement (Figure II). Best's glycogen stain on formalin-fixed, paraffin-embedded tissues (Vallance-Owen, 1948) showed an abundance of glycogen granules in many hepatic cells, although the individual cellular content varied considerably. There was an increase in cells showing fatty vacuolation and a marked increase in portal connective tissue. The cells in the nodule did not differ appreciably from those in the rest of the organ except that they were more vacuolated, with more fat and glycogen. There was less connective tissue in this area.

The pancreas showed only a few, rather small islets, but was otherwise normal.

The kidneys showed much glycogen in the convoluted tubules, where the cells were swollen and vacuolated, often protruding into the lumen, producing a scalloped effect (Figure III). The cellular débris in the lumen also contained some glycogen. Most of the loops of Henle showed similar but less pronounced changes. The collecting tubules were normal except for a few with flattened tubular epithelium and casts. Mallory and Masson stains showed much increase in interstitial fibrous tissue. The glomerular capillaries were thickened by eosinophilic hyaline material deposited both diffusely and as focal masses (Figures IV and V). These latter were circular or crescentic in shape, varying in size from rings barely surrounding a capillary to nodular masses occupying one-third of a glomerulus. Cells were usually, though not invariably, present, both at the centre and at the periphery of these lesions. Centrally the cells were, as a rule, recognizable as parts of a capillary wall, while peripherally they formed a single layer around the masses, quite distinct from Bowman's capsule. The lesions stained green with the Masson trichrome procedure, blue with Mallory's triple stain, red with phosphotungstic acid hæmatoxylin and pink with Van Gieson's stain. They gave a negative result with fat stains. Approximately one in four glomeruli showed these lesions. A few glomeruli

FIGURE III

Swollen epithelium of convoluted tubules with normal collecting tubules. Hæmatoxylin and eosin. (×220)

contained, in addition, globular, acellular, brightly eosinophilic masses giving the staining reactions of fibrinoid material. (See Figure IV.)

The arterioles showed hyaline degeneration with luminal narrowing but no evidence of fibrinoid necrosis. The other organs did not show any significant changes.

ENZYME STUDIES

Glucose-6-phosphatase estimations were performed on liver homogenates from this patient and a normal control according to the method of Cori and Cori (1952). The control liver came from a seventy-two-year-old man who had died of paralytic ileus following the removal of a normal appendix. In both cases the interval between death and freezing of the tissue to −20° C. was twenty-two hours, and enzyme estimations were performed within three months. The results are shown in Table I. It will be seen that the glucose-6-phosphatase in the normal liver accounted for 310 microgrammes of inorganic phosphorus per 100 milligrammes dry weight, while the patient's liver liberated only 50 microgrammes

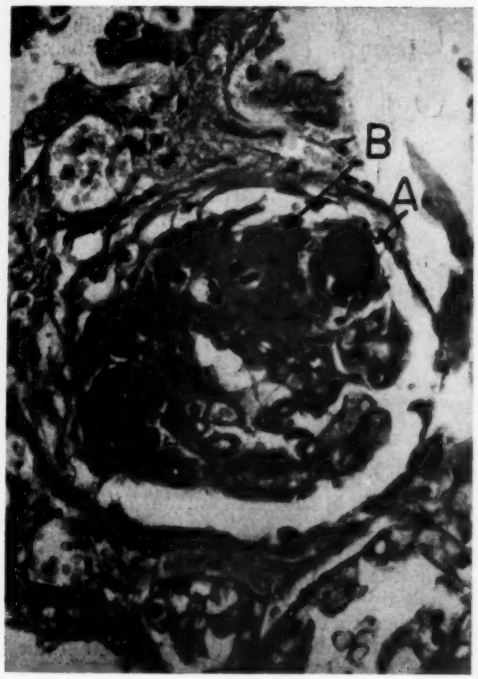

FIGURE IV

A mixture of " hyaline fibrinoid " (A) and " nodular " lesions (B). Hæmatoxylin and eosin. (×340)

of inorganic phosphorus per 100 milligrammes of dry weight.

Lactic dehydrogenase activity was determined in both patient's liver and that of the normal control according to the method of Kornberg (1955). This was done to establish whether other enzymes were also diminished in the diseased organ. No such diminution was found. Indeed, lactic dehydrogenase activity was identical in the two specimens (11 units of enzyme activity per 100 milligrammes dry weight of liver).

FIGURE V

The glomerular lesions as shown by the periodic acid-Schiff stain. (×220)

GLYCOGEN STUDIES

A part of the patient's liver weighing 5·3 grammes was treated according to the method of Polglase, Smith and Tyler (1952). The glycogen yield was 0·25 gramme (4·7%) As the normal liver used as a control in the enzyme estimations was expected to yield little or no glycogen, livers from two adult male Wistar rats, which had been fed the ordinary laboratory diet, were used as controls. The animals were killed in the gas chamber and their livers removed and frozen within two minutes. Rat liver weighing 10·4 grammes yielded 0·44 gramme of glycogen (4·4%).

When dissolved, the patient's glycogen was more opalescent than that of the rat. Optical rotations were $+190° \pm 10°$ for the patient's glycogen and $+180° \pm 10°$ for the rats' glycogen.

Ultracentrifugation was performed on the two samples of glycogen by Mr. D. J. Winzor, Department of Physical Chemistry, University

of Adelaide, according to the method of Polglase, Brown and Smith (1952). Sedimentation patterns are shown in Figure VI. The $S_{20,w}$ values were 69 S for the patient's glycogen and 74 S for the rat glycogen. It will be seen from the first photograph in Figure VIA that

TABLE I

Results of Incubating Patient's Liver and Normal Control with and without Glucose-6-Phosphate

Material Incubated	Inorganic Phosphorus Liberated in Microgrammes per Millilitre of Reaction Mixture[1]
Normal liver without substrate	38
Normal liver with substrate	100
Difference (accounted for by glucose-6-phosphatase activity)	62
Patient's liver without substrate	27
Patient's liver with substrate	37
Difference (accounted for by glucose-6-phosphatase activity)	10

[1] Each millilitre of reaction mixture contained 20 milligrammes dry weight of liver (=66 milligrammes wet weight).

a slight trace of a heavier component was also present in the patient's glycogen. This is the usual finding in hepatic glycogen from normal individuals. In the few cases of Von Gierke's disease with ultracentrifugation studies reported by Polglase, Brown and Smith (1952) and by Zetterström and Sörbo (1956) this heavy component was previously found to be absent.

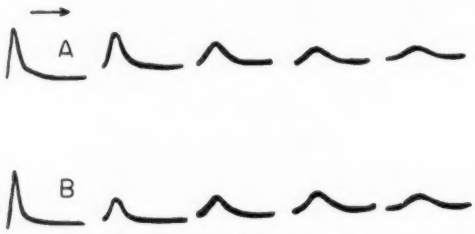

FIGURE VI (slightly retouched)

Sedimentation pattern of patient's glycogen (A) and rat glycogen (B). A 1% solution in 1M sodium chloride centrifuged at 20,410 revolutions per minute. Eight minutes' interval between exposures

One hundred milligrammes of the patient's glycogen were sent to Dr. G. T. Cori, of St. Louis, United States of America, for structure analysis. She found the molecular structure of the submitted material to be identical with that of normal glycogen.

DISCUSSION

The various types of glycogen storage disease have recently been the subject of an excellent review (Recant, 1955). Type I, also known as Von Gierke's disease, is characterized by the deposition of abnormal amounts of structurally normal glycogen in the liver and kidneys. The underlying metabolic disturbance is a deficiency of glucose-6-phosphatase. In Type II (Pompe's disease) glycogen is deposited in the heart, skeletal musculature, tongue and numerous other organs. Death occurs in early infancy, usually from congestive heart failure. No enzymatic deficiencies or abnormalities of glycogen structure have been demonstrated in this disease. Types III and IV are associated with demonstrable abnormalities of glycogen structure. In Type III there is a deficiency of the "branching" enzyme, so that these patients have glycogen with long chains and few branching points. The abnormal glycogen is taken up by the reticulo-endothelial system. Type IV is due to a deficiency of the debranching enzyme, amylo-1,6-glucosidase (Illingworth *et alii*, 1956). The result is an abnormal glycogen with numerous short side chains.

The presence in our patient of normal amounts of lactic dehydrogenase strongly suggests that the decrease in glucose-6-phosphatase activity was not due to the displacement of liver tissue by inert material (glycogen, fat, fibrous tissue) or to post-mortem changes. This deficiency of glucose-6-phosphatase and the restriction of glycogen accumulation to those parts of the body normally containing this enzyme leave little doubt that this was, in fact, a case of Type I glycogen storage disease (Cori, 1952–1953). Cases are on record (Evans *et alii*, 1955) in which glycogen accumulation has been associated with severe *diabetes mellitus*. The absence of glycosuria in this patient and the fact that he lived for thirty-one years without insulin would make this possibility a remote one, in spite of the small amount of islet tissue in his pancreas.

The renal tubular lesions were to be expected. While it is tempting to attribute the symptoms of polydipsia and polyuria in this patient and his father to tubular dysfunction, such ideas must, in the absence of detailed renal studies, remain speculative. Certainly, the finding of a low urinary specific gravity on one occasion (in the presence of a brain-stem lesion) does not constitute proof of insufficient tubular reabsorption. It is interesting to note that Crawford's (1945) third patient always drank "a lot of water", but did not pass "excessive" amounts of urine.

Aneurysms of the renal artery can cause hypertension (Burns, 1956). In the few reported cases in which this cause and effect has actually been demonstrated, some degree of vascular

obstruction was invariably present. No such obstruction was found in the present case. It would, therefore, appear more likely that the renal artery aneurysm was due to a congenital deformity and/or the result of the patient's hypertension rather than its cause.

Most significant of all are the glomerular lesions, which closely resemble intercapillary glomerulosclerosis described by Kimmelstiel and Wilson (1936). The rarer, brightly enosinophilic masses are identical with the hyaline fibrinoid lesions of Koss (1952), while the common cellular masses resemble the nodular glomerular lesions described by Koss (1952) and the cellular hyaline lesions described by Muirhead *et alii* (1956). Nodular glomerulosclerosis, particularly if extensive, is almost diagnostic of *diabetes mellitus*. Its occurrence in association with a disease characterized by hypoglycæmia therefore needs some explanation.

There are certain features common to both disorders of carbohydrate metabolism (Van Creveld, 1939). The high levels of some serum lipoproteins in glycogen storage disease resemble those of uncontrolled diabetes (Kolb *et alii*, 1955). If, as has been suggested (Engelberg *et alii*, 1952) there is any relationship between abnormality of serum lipids and lipoproteins and the development of glomerulosclerosis in diabetes, a similar association is to be expected in glycogen storage disease. In at least one of the recorded cases of Von Gierke's disease with severe hyperlipæmia (Zakon *et alii*, 1953) there was mild albuminuria with a few white cells and casts in the sediment.

There is, at present, no proof that disordered lipid metabolism bears any relation to the development of diabetic glomerulosclerosis. Other metabolic abnormalities may ultimately be shown to be responsible for the lesion in diabetes and related disorders.

ACKNOWLEDGEMENTS

My thanks are due to Dr. P. M. Nossal and other members of the Biochemistry Department for their help with the enzyme estimations, to Dr. W. Roman, of the Institute of Medical and Veterinary Science, for help with the glycogen preparation, to Professor D. O. Jordan for use of the ultracentrifuge, to Miss M. Dopson for assistance with the photographs, and to Miss F. Vogt for preparation of the sections. I am also indebted to Dr. B. Nicholson for permission to use the clinical notes and to Dr. M. Fowler for his help in preparing the manuscript.

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HÆMOGLOBIN AS A SOURCE OF IRON IN NUTRITION

SOME IN-VITRO EXPERIMENTS[1]

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SUMMARY

Hydrochloric acid and pepsin can liberate little of the iron contained in native hæmoglobin; but if hæmoglobin is denatured by heat, prior to the application of these agents, the yield of iron is increased several fold.

ON contact with hydrochloric acid, native hæmoglobin is immediately converted into acid hæmatin, a compound which contains the iron in a firm linkage and which is highly stable. Presumably the same reaction occurs in the stomach upon ingestion of blood. For this reason it has been considered in the past that the iron of hæmoglobin is not available for absorption, and therefore that hæmoglobin is of almost no value as a source of dietary iron (Drabkin, 1951).

It was, however, reported recently that ingestion of cooked sheep's blood by human subjects resulted in the absorption of an appreciable portion of the hæmoglobin iron (Walsh *et alii*, 1955). In an attempt to explain this finding, the liberation of iron from hæmoglobin was studied *in vitro*. The results reported in this paper show that, while peptic digestion can liberate only a small amount of iron from native hæmoglobin, the amount liberated is greatly increased if hæmoglobin is denatured by cooking prior to digestion.

MATERIALS AND METHODS

Materials

Blood Samples.—Blood was obtained from human volunteers. Approximately 50 millilitres were collected into a flask containing a small amount of potassium oxalate as anticoagulant. Unless otherwise stated, the blood was used within twelve hours of collection. The hæmoglobin content of each sample was determined on three replicate aliquots by the method of Walsh *et alii* (1953) and the iron content was calculated using Hüfner's figure (0·334%) for the iron content of hæmoglobin.

Physiological Saline.—A 0·9% solution of sodium chloride in distilled water was used.

Hydrochloric Acid.—A 3·2N solution of hydrochloric acid in distilled water was used as stock solution.

Pepsin.—Commercial pepsin powder conforming to specifications of the British Pharmacopœia (Parke, Davis "Pepsin" 1 : 2500) was kept in a desiccator vessel. Where pepsin was required in the reaction, aliquots were weighed immediately before use and added in the powder form.

Experimental Procedure

Preparation of Hæmoglobin.—One millilitre of blood was pipetted into a 25-millilitre volumetric flask containing 10 millilitres of distilled water or saline. Where the object of the experiment was to test the effect of preliminary heat denaturation on the liberation of iron, the flask containing the diluted blood was immersed in a 95° C. water bath for varying periods. During the first five minutes of immersion the flask was agitated.

The flask was made up to volume with distilled water or saline, depending on the method of preparation. Where required, hydrochloric acid and pepsin were added to the flask before making up to volume. The flask was then placed in a water bath of appropriate temperature. The concentration of acid and of pepsin and the duration and temperature of hydrolysis are given in Table I.

It should be noted that throughout this paper the word "hydrolysis" is used in a loose sense. Hæmoglobin, as a protein, certainly undergoes hydrolytic decomposition by acid and

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TABLE I

The Liberation of Iron from Hæmoglobin

Experiment Number	Hæmoglobin Preparation	Conditions of Hydrolysis		Percentage of Iron Liberated					
				From Hæmoglobin not Heated before Hydrolysis			From Hæmoglobin Heated to 95° C. before Hydrolysis[1]		
		Temperature (°C.)	Duration (Minutes)	In Neutral Medium[2]	In Dilute Hydrochloric Acid[3]	In Dilute Hydrochloric Acid Containing Pepsin[4]	In Neutral Medium[2]	In Dilute Hydrochloric Acid[3]	In Dilute Hydrochloric Acid Containing Pepsin[4]
1	Hæmolysed blood..	90	30	0·6	3·4	—	0·4	10·6	—
2	Hæmolysed blood..	90	30	—	3·5	—	—	10·8	—
		40	30	—	1·9	—	—	8·1	—
3	Hæmolysed blood..	40	30	—	3·8	—	—	7·7	—
	Saline-diluted blood			—	2·1	—	—	7·6	—
4	Saline-diluted blood	37	60	—	2·4	1·8	—	7·6	11·4
5	Saline-diluted blood	37	60	—	—	—	—	—	11·0–12·5 (4 replicates)
	As above, stored five days at 6° C.			—	—	—	—	—	12·4–12·7 (2 replicates)
6	Saline-diluted blood	37	60	—	—	2·4	—	—	11·4–11·6 (2 replicates)
		37	120	0	—	2·9	1·4	—	12·1–12·4 (2 replicates)
7	Saline-diluted blood	37	60	0·1	—	0·4–1·1 (3 replicates)	3·0	—	11·5–13·9 (3 replicates)

[1] Duration of heating: Experiments 1–5, 30 minutes; Experiment 6, 30 and 60 minutes for different replicates; Experiment 7, 5, 15 and 30 minutes for different replicates.
[2] Distilled water or saline, depending on preparation.
[3] 0·16N to 0·32N hydrochloric acid solution.
[4] 0·32% to 0·64% pepsin (Pepsin B.P.) in 0·16N to 0·32N hydrochloric acid solution.

pepsin, but the role of hydrolysis in the liberation of iron has not been investigated.

Extraction and Assay of the Liberated Iron.—On completion of hydrolysis the contents of the flask were transferred to a tube and centrifuged at 2000 *g* for thirty minutes. An aliquot of 10 millilitres of the supernatant solution was pipetted into a graduated centrifuge tube. One millilitre of a 4% solution of trichloracetic acid was added and the tube placed in a 90° C. water bath for fifteen minutes. The tube was then centrifuged at 2000 *g* for fifteen minutes.

Iron assay was performed on duplicate aliquots of the supernatant solution obtained. An absorptiometric method using 1 : 10 orthophenantroline reagent, described earlier for the determination of iron extracted from serum, was adapted for this purpose (Kaldor, 1953). Blank solutions containing all reagents but no blood were used.

RESULTS

The results shown in Table I demonstrate (*a*) that native hæmoglobin yields very little of its iron when hydrolysed with acid and pepsin, and (*b*) that the process of heating the hæmoglobin prior to hydrolysis does not of itself liberate much iron from hæmoglobin, but in some manner facilitates the action of acid and pepsin. This result remains essentially the same whether the blood is prepared in saline or in distilled water, and it is little affected by the temperature or duration of hydrolysis, by variations in the concentration of acid and pepsin, or by using blood after five days' storage at 6° C. It will be noted that in Experiments 1 and 2 the temperature of hydrolysis was 90° C. These experiments were included because this temperature is used in the acid hydrolysis of animal tissues for the estimation of non-hæmatin iron (Kaldor, 1954).

It appears from Experiment 7 that five minutes' preliminary heating is sufficient to reduce the resistance of the hæmoglobin molecule to the action of acid and pepsin.

DISCUSSION

The results show that denaturation of hæmoglobin by heat, such as must occur in the process of cooking, reduces the apparent

resistance of native hæmoglobin to removal of its iron by treatment with dilute hydrochloric acid or by peptic digestion. The only alternative to this interpretation of the reported results is that iron released from hæmoglobin in the course of hydrolysis with acid-pepsin is adsorbed to the surface of the globin moiety, and that adsorption is more complete if the globin is not denatured by heat prior to hydrolysis. This possibility, however, was explored in preliminary experiments, where known amounts of iron were added to both native and heated hæmoglobin, and the recovery of the added iron was measured. No differences were found.

It would appear that the present findings provide an explanation for the absorption of hæmoglobin iron from cooked sheep's blood by the subjects of Walsh *et alii* (1955). These subjects absorbed 2% to 4% of the iron of ingested hæmoglobin. In the present work, 12% to 13% of the iron was liberated from heat-denatured hæmoglobin by peptic digestion *in vitro*. If it is assumed that a similar portion of the hæmoglobin iron was liberated from cooked blood in the stomachs of the subjects, the amounts absorbed would represent one-sixth to one-third of the iron liberated from hæmoglobin. These proportions are somewhat higher than one would expect under normal conditions, but it must be pointed out that one week before the subjects ingested the cooked blood, 500 millilitres of blood had been withdrawn. Their physiological demand for iron would therefore have been higher than normal.

The possibility that peptic digestion is not the only mechanism responsible for the liberation of iron from cooked blood in the gastro-intestinal canal must also be considered. It has been suggested that microorganisms of the intestinal flora may play a part in the liberation of iron from hæmoglobin.

ACKNOWLEDGEMENTS

The author wishes to acknowledge the technical assistance of Miss Marjorie Powell.

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HÆMOGLOBIN AS A SOURCE OF IRON IN NUTRITION[1]

THE ABSORPTION OF IRON FROM Fe^{59}-LABELLED HÆMOGLOBIN BY RATS

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SUMMARY

The absorption of iron from hæmoglobin introduced into the gastro-intestinal tract was investigated in rats. It was found that a greater amount of iron was absorbed from heat-denatured hæmoglobin than from hæmoglobin administered in its native state. Approximately half as much was absorbed from hæmoglobin administered into the jejunum as from hæmoglobin administered into the stomach.

IT has been shown that human subjects can absorb iron from cooked hæmoglobin (Walsh *et alii*, 1955). As there is no evidence that the hæmatin molecule can be absorbed as such, it was concluded that iron must be liberated from the hæmoglobin molecule in the gastro-intestinal canal before absorption. In-vitro experiments have shown that preliminary heating enables acid and pepsin to liberate considerable amounts of iron from the hæmoglobin molecule, but heating does not of itself produce this effect (Kaldor, 1957).

The present work was undertaken primarily to investigate *in vivo* whether a greater amount of iron is absorbed from hæmoglobin which has been heated than from hæmoglobin in its native state. The opportunity was also taken to investigate the amount of iron absorbed from hæmoglobin when injected into the jejunum.

METHODS AND MATERIALS

Plan of Experiment

Blood labelled with Fe^{59} was obtained from donor rats injected with a solution of $Fe^{59}Cl_3$. The red cells were separated and divided into two portions. One portion was heated to simulate the process of cooking, whilst the other portion was not treated. In the remainder of this paper the heated preparation is referred to as "denatured hæmoglobin" and the unheated preparation as "native hæmoglobin". These were administered to four groups of rats as follows:

Group I. Native hæmoglobin into the stomach.
Group II. Denatured hæmoglobin into the stomach.
Group III. Native hæmoglobin into the jejunum.
Group IV. Denatured hæmoglobin into the jejunum.

After nineteen days the rats were killed, blood was collected from the heart, and the liver and spleen were removed. The radioactivity of the blood and organs was assayed, and the amount and percentage of iron absorbed from the ingested material was calculated.

Radioactive Iron Solution

Radioactive iron (Fe^{59}) in the form of ferric chloride solution was obtained from the Atomic Energy Research Establishment, Harwell, through the agency of the Commonwealth X-Ray and Radium Laboratory, Department of Health, Australia. The solution of five millilitres had an iron content of 0·842 milligramme per millilitre and an activity of 21 microcuries per millilitre at the time of injection.

Preparation of Labelled Blood

Five male albino rats were each injected with one millilitre of the radioactive iron solution. The dose was divided into two portions, which were injected into the femoral veins on successive days. Nineteen days later blood was obtained

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TABLE I

Absorption of Hæmoglobin Iron

Group	Number of Rats	Mean Body Weight (Grammes)	Hæmoglobin Preparation and Route of Administration	Dose of Hæmoglobin Iron Administered per Rat (Milligrammes)	Iron Absorbed		
					Total Found in Blood, Liver, and Spleen		Total Found in Liver and Spleen as Percentage of Dose
					As Percentage of Dose	Microgrammes ±Standard Error of Mean[1]	
I	8	355	Native, into stomach	0·53	0·80	4·19 ±0·265	0·06
II	8	377	Denatured, into stomach	0·38	1·88	7·14 ±0·920	0·13
III	8	351	Native, into jejunum	0·48	0·37	1·78 ±0·314	0·04
IV	6	304	Denatured, into jejunum	0·33	1·35	4·41 ±0·790	0·12

[1] Standard error of mean is obtained from $\frac{\sigma}{\sqrt{n}}$ when n is the number of observations.

from these rats. Under light ether anæsthesia the chest was opened in the mid-line and blood collected from the heart with a needle and syringe containing heparin. Approximately 40 millilitres of blood were obtained and pooled in a bottle containing potassium oxalate as anticoagulant.

Preparation of Hæmoglobin for Administration

The blood was centrifuged, the supernatant was discarded, and the centrifuged red cells were divided into two aliquots of 11·5 millilitres and 13·5 millilitres.

The smaller aliquot of 11·5 millilitres was suspended in physiological saline to give a total volume of 36·5 millilitres.

Approximately 25 millilitres of physiological saline were added to the larger aliquot, and the suspension was placed in a water bath at 100° C. for fifteen minutes. During this time the material was stirred. The resulting coagulated material was transferred to a wet grinder and homogenized with saline. The final volume of the suspension obtained was 36 millilitres.

The quantities of blood and diluents used in the preparation of these suspensions were designed to allow for losses during the heating and homogenizing of the denatured preparation. As will be seen later, the losses were greater than had been anticipated.

Administration of Hæmoglobin

Suspensions were injected through a thin polythene tube passed into the stomach. Two millilitres of suspension were administered from a syringe, and the tube was washed with two millilitres of saline.

Suspensions were injected into the jejunum immediately above the root of the mesentery. The rats were anæsthetized, the abdominal cavity was opened, and two millilitres were injected through a needle.

Collection and Radioassay of Specimens

Nineteen days after administration of labelled hæmoglobin, the animals were killed, blood was collected from the hearts, and the livers and spleens were removed after perfusion with saline. Specimens were prepared for radioassay as described previously (Walsh *et alii*, 1955), and the radioactivity was measured in a scintillation counter adapted for counting liquid samples.

Calculation of Results

The iron content of the injected doses of hæmoglobin was determined as follows: The iron content of the blood obtained from donor rats was calculated from the mean hæmoglobin concentration of similar rats (15·6 grammes per 100 millilitres—Kaldor and Powell, 1957) and 0·334% for the iron content of hæmoglobin. From this the iron content of native hæmoglobin was calculated, taking into account the dilution used in preparation of the final solution. The iron content of the heat-denatured hæmoglobin was determined from the relative radioactivity found in one-millilitre aliquots of native and of denatured hæmoglobin and the iron content of the native hæmoglobin.

The percentage of the dose absorbed by each rat was obtained, as previously described (Brading *et alii*, 1956), from the radioactivity of the dose and the radioactivity found in the

body. From this percentage and the iron content of the dose, the amount of iron absorbed was calculated.

RESULTS

The results are shown in Table I. The amounts of iron absorbed and found in the blood, liver and spleen combined are given as percentages of the doses administered and as absolute amounts. From the last column of the table, it is seen that only about one-tenth of the iron absorbed was found in the liver and spleen.

From Groups I and II it is clear that heating enabled a greater amount of iron to be absorbed from hæmoglobin. It should be noted that this occurred although the amount of iron administered as denatured hæmoglobin was less than when hæmoglobin was given in its native state. In those animals which received the hæmoglobin by injection into the jejunum the effect of preliminary heat denaturation is again seen.

The difference in absorption from the stomach and from the jejunum is seen by comparing results of Group I with Group III, and Group II with Group IV. Less iron was absorbed when the hæmoglobin was administered into the jejunum, but the differences were not as great as expected.

DISCUSSION

The present work shows that preliminary heat denaturation of hæmoglobin enables a larger amount of iron to be absorbed than when the hæmoglobin is administered in the uncooked state. This confirms in-vitro experiments (Kaldor, 1957), in which it was shown that cooking facilitates the liberation of iron from the hæmoglobin molecule. The percentages and amounts of iron absorbed, as shown in Table I, are relatively small. However, these small values can probably be explained by the fact that only a portion of the hæmoglobin iron was available for absorption. In the in-vitro experiments 2·5% of hæmoglobin iron was liberated by acid pepsin digestion from uncooked blood and 12·5% from cooked blood. If these figures are applied to the present work, it may be assumed that the dose of available iron administered to the animals in Group I was 0·013 milligramme and in Group II 0·048 milligramme. The percentage of this liberated iron absorbed by the animals in Group I was 32% and by the animals in Group II 15%. These percentages are similar to those obtained from equivalent doses by Brading *et alii* (1956), when different doses of inorganic iron salts were administered to rats. This suggests that the in-vivo liberation of iron from cooked and uncooked blood is of the same order as that found in the in-vitro experiments.

The amount of iron absorbed when hæmoglobin was injected into the jejunum was unexpectedly large compared with that absorbed when hæmoglobin was administered into the stomach. The chemical *milieu* of the intestinal canal is very different from that of the stomach, and it is not known whether any iron is liberated here from hæmoglobin. It is possible that a portion of the injected hæmoglobin regurgitated into the duodenum and stomach, and that iron was liberated and absorbed in these regions.

From the results reported in this paper it is clear that patients who suffer from gastro-intestinal hæmorrhage absorb very little of the hæmoglobin iron. The iron deficiency afte such hæmorrhage is easily understood as a result of these findings. On the other hand, hæmatin compounds (hæmoglobin, myoglobin and hæmatin enzymes) in food which has been cooked may contribute significant amounts of iron to an iron-deficient patient. It should be pointed out that in the experiments reported above the animals used were at no time iron-deficient. A considerably greater quantity of iron would probably have been absorbed if iron deficiency had been present (Hahn *et alii*, 1943).

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ADDENDUM

Since this paper was submitted, Sheila T. Callender, Barbara J. Mallett and Mary D. Smith (*Brit. J. Hæmat.*, April, 1957, **3**, 186) have reported measurements on the absorption of iron from hæmoglobin ingested by human subjects. They found that hæmoglobin iron was absorbed, but that heat denaturation did not increase the absorption.

PRIMARY ALDOSTERONISM[1]

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SUMMARY

A female patient, aged thirty-one years, suffering from primary aldosteronism due to adrenal adenoma (Conn's syndrome) is described. She presented with hypertension of the order of 240/120 millimetres of mercury associated with weakness, polyuria up to five litres a day, and an electrocardiogram showing *ST* depression and prominent *U* waves in various leads. The serum potassium content was lowered, and there was marked alkalosis with a failure to concentrate urine. The urine contained aldosterone in concentration of 10 to 11 microgrammes per litre, and the plasma aldosterone level was approximately 2·8 microgrammes per 100 millilitres.

A left suprarenal tumour, demonstrated by presacral air insufflation, was removed at operation. The hypertension and metabolic abnormalities reverted to normal.

The alkalosis appeared to be secondary to the hypokalæmia and was partly corrected by administration of potassium citrate. Observations suggested that the defect in urine concentration was due to antagonism to antidiuretic hormone.

Methods used for estimating mineralo-corticoid activity of plasma and urine are outlined.

THE elegant work that led to the recognition of the clinical state that results from the excessive secretion of aldosterone is now well known. It is indeed a triumph that Conn and his colleagues (Conn, 1955) were able to describe this syndrome so shortly after the isolation of aldosterone by Tait *et alii* (1952).

Since Conn's original description, a number of patients with primary aldosteronism have been reported (Mader and Iseri, 1955; Cope and Milne, 1955; Chalmers *et alii*, 1956; Van Buchem *et alii*, 1956; Campbell *et alii*, 1956). Most of these patients have presented with hypertension and the effects of lowered serum potassium content. The recognition of this syndrome is of importance because the hypertension and the other metabolic abnormalities can usually be ameliorated by a simple surgical procedure.

We record here our findings in a patient suffering from this disorder, including observations on the disturbance of water and mineral metabolism, and describe the methods used for the isolation of aldosterone from the plasma and urine.

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CASE REPORT

Mrs. M.C., a housewife, presented for treatment at another hospital during the latter part of 1954, when aged thirty-one years. She complained of frontal headaches and tiredness of recent onset, and her blood pressure was found to be raised. She was admitted to that hospital for further investigation and treatment in February, 1955. Injection of phentolamine ("Regitine", Ciba) produced a fall in blood pressure insufficient for a diagnosis of phæochromocytoma. Treatment with pentolinium ("Ansolysen", May and Baker) by injection failed to reduce her blood pressure or relieve her symptoms, which, in fact, became worse.

Because of this unsatisfactory response she was referred to this hospital and was first seen by one of us (A.J.B.) in June, 1955. At that time she was complaining of frontal headaches, weakness, shortness of breath on exertion, attacks of giddiness and the fact that she was passing large volumes of urine twice during the night. She also complained of episodic weakness, although not of frank paralysis. She was overweight (157 pounds) and slightly hirsute, and a casual blood pressure reading was 240/120 mm. Hg. Investigation as an out-patient revealed findings in accord with the diagnosis of benign hypertension. An electrocardiogram indicated ventricular hypertrophy.

As, at that time, a trial of the effectiveness of reserpine and of reserpine with the addition of hydralazine was in progress, the patient received, in turn, treatment with a placebo preparation, with reserpine *plus* hydralazine and with reserpine alone. Neither her symptoms nor her hypertension were relieved by these treatments. In addition to her other complaints, she noted that her thirst had become excessive and her lethargy more marked. This was regarded at first as a possible side-effect of reserpine, but became more severe than

in other patients being treated with this drug. Because of this, and the complete lack of response to therapy, it was considered advisable to stop this treatment and admit her to hospital for further investigation.

Her past history and family history gave no indication as to the cause for her hypertension. Casual observations of her blood pressure during 1949 and 1950 had been 130/80 and 100/65 mm. Hg respectively.

On admission to the Alfred Hospital her casual blood pressure was 210/110 mm. Hg. The cardiac apex was in the fifth left interspace, five and a half inches from the mid-line. The heart sounds were normal. There was no elevation of jugular venous pressure; nor was there any œdema. Her breath sounds were normal. Examination of her abdomen revealed obesity but no other abnormality. Trousseau's sign was positive. The optic fundi showed narrowed arteries of varying calibre, with minor nipping of the veins at some of the arterio-venous crossings, but no other changes. The urine was alkaline to litmus, contained no albumin, and had a specific gravity of 1005. An electrocardiogram (Figure I) taken shortly after admission showed significant *ST* segment depression in leads I, II, V5, V6 and V7 and flattening of the *T* wave in the standard and unipolar limb leads and the precordial leads V4 to V7. The tracing also showed a conspicuous *U* wave in leads V3R to V4 with a voltage approximating that of the *T* wave in V4. QT_c was increased (0·47 second). There was no evidence of ventricular hypertrophy. These abnormalities were interpreted as being due to the effects of lowered blood potassium content.

Plasma electrolytes had the following concentrations at the same time (in milliequivalents per litre): sodium, 147; potassium, 2·1; calcium, 5·5; magnesium, 1·2; chloride, 97·0; bicarbonate, 39·0; protein, 18·0; phosphate, 1·4.

Subsequent investigations showed that renal function, when measured by the conventional urea concentration-excretion method, was not impaired: after 15 grammes of urea she was able to concentrate to more than 2%, and she excreted 8·3 grammes of urea in three hours; urea clearance was 110% of average normal. More than 80% of a 1500-millilitre water load was excreted in the subsequent four hours, but after fluid deprivation for twelve hours she was unable to concentrate to a greater specific gravity than 1016. The urine pH values (glass electrode) were persistently alkaline, and ranged between 7·2 and 7·6. Her glucose tolerance was normal, as was the result of hæmatological examination. X-ray examination of her chest showed a moderate degree of cardiac enlargement with some pulmonary congestion. The pH of an arterial blood sample was 7·55. Studies of the salivary sodium/potassium ratio were undertaken, but these yielded inconclusive results in that her value was within the range of values given by seven other normal persons studied simultaneously.

At this stage it was decided that this patient presented many of the features of primary aldosteronism, and investigations were directed accordingly. Her twenty-four-hour output of urinary steroids was as follows: 17-hydroxysteroids, 9·4 to 11·8 milligrammes (normal, 5 to 10 milligrammes); 17-ketosteroids,

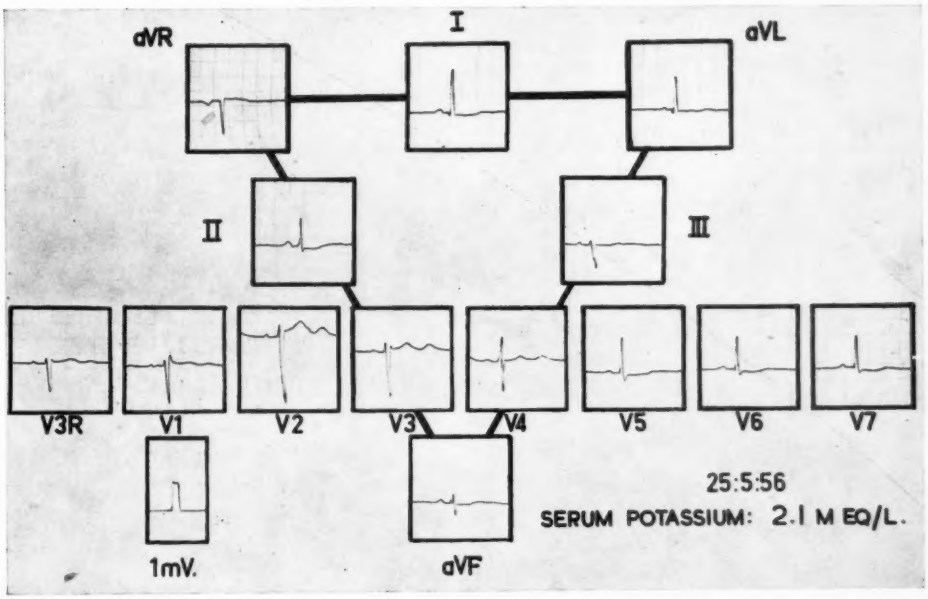

FIGURE I

An electrocardiogram taken shortly after the patient's admission to hospital showing *ST* segment depression in leads I, II, V5, V6 and V7 with *T* wave flattening and a conspicuous *U* wave in leads V2, V3 and V.4 The serum potassium level was 2·1 milliequivalents per litre

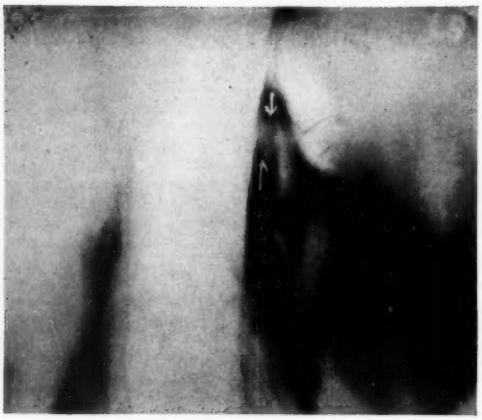

FIGURE II

Presacral air insufflation. Tomogram (antero-posterior) at nine centimetres in the region of the upper pole of the left kidney. A rounded shadow (indicated by arrows) may be seen on the upper edge of the left adrenal

FIGURE III

Presacral air insufflation. Tomogram (lateral) at 17 centimetres with patient on left side. A rounded shadow (indicated by arrow) may be seen above the renal and behind the splenic shadows

FIGURE IV

The adrenal tumour exposed at operation. The upper border of the adrenal (A) may be seen to the left of the tumour (T)

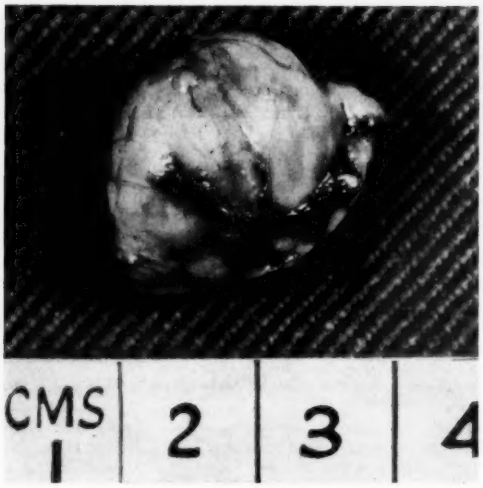

FIGURE V

The tumour after removal. Its discrete nature may be seen from the cut surface

9·8 to 14·7 milligrammes (normal, 8 to 12 milligrammes). Her plasma steroid concentrations were as follows: 17-hydroxysteroids, 9·6 microgrammes per 100 millilitres (normal, 10 to 15 microgrammes per 100 millilitres); aldosterone, 2·8 microgrammes per 100 millilitre (approximately).

An extract of a five-day specimen of urine (approximately 25 litres) subjected to chromatography had an aldosterone concentration of between 10 and 11 microgrammes per litre.

An intravenous pyelogram was normal. Presacral air insufflation (one litre of air) showed a small rounded opacity, about 1·5 centimetres in diameter, above the upper pole of the left kidney in both the postero-anterior and lateral tomograms (Figures II and III) This was interpreted as being due to a discrete tumour of the left adrenal gland.

FIGURE Va

The tumour after removal. Its discrete nature may be seen from the cut surface

In view of the clinical, biochemical and radiological findings, the region of the left adrenal gland was explored surgically (Professor M. R. Ewing). At operation, a spherical tumour measuring approximately two centimetres in diameter was found attached to the left adrenal gland. Its surface was smooth and its consistency soft. The tumour was mobilized and removed by cutting across the pedicle (Figures IV and V).

Dr. A. V. Jackson reported on the tumour as follows:

> Microscopically, the tumour consists of cells with small, regular and usually eccentric nuclei and abundant, foamy, eosinophilic protoplasm. These cells are arranged in small groups, comprising three to six cells, distinctly separated by a very vascular stroma. The cells resemble the normal cells of the adrenal cortex and the pattern of arrangement is similar to that of the *zona glomerulosa*. There is nothing in the appearance of these cells as shown by hæmatoxylin and eosin or Masson staining by which this tumour may be distinguished from any other benign adrenal cortical adenoma.

Post-operatively, the patient presented no problems. The blood pressure gradually fell to lower levels, so that on her discharge from hospital one month after surgery it ranged between 120/80 and 170/110 mm. Hg.

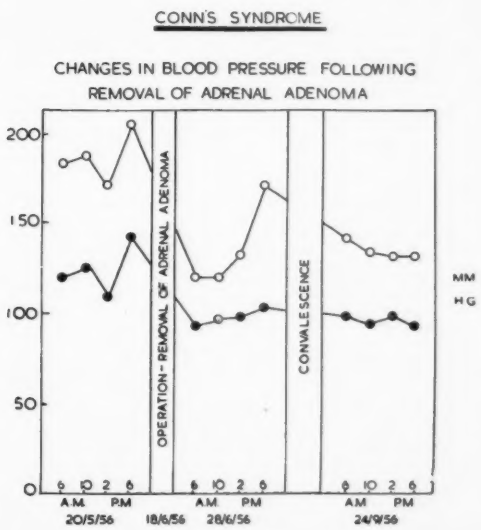

FIGURE VI

Showing four-hourly blood pressure recordings during a day before operation, shortly after and three months after removal of the adrenal tumour

Her feelings of weakness and lethargy had disappeared. She was still troubled by headaches, although these were less severe. Trousseau's sign was negative.

The values of serum electrolytes gradually returned to normal. The arterial pH was 7·41 on the twelfth post-operative day, and the urine pH values were between 5·3 and 6·6 (glass electrode). The urine volumes had diminished from the previously high level to between one and a half and two litres per day, and there was an associated decrease in thirst. After water deprivation the patient was now able to concentrate to a specific gravity of 1024.

Although urinary steroid values after operation were of the same order as those before operation, aldosterone was not detected in a five-day urine specimen; nor could aldosterone be detected in her plasma.

In September, 1956, the patient was readmitted to hospital for further studies. At this stage she was still troubled by frontal headaches, but felt that they were somewhat less intense than before operation. The tiredness and weakness of which she complained before operation had completely disappeared, so that now she was able to perform her housewifely duties with more enthusiasm. Her weight was unchanged. She appeared more alert and interested than before operation, although on one occasion she was observed in a moderately depressed state. Her blood pressure ranged between 160/100 and 120/80 mm. Hg when observed four-hourly during the day. The changes in blood pressure are shown in Figure VI. Her heart size was normal to clinical examination. Trousseau's sign was negative.

While she was in hospital her urine volumes ranged between 1100 and 1900 millilitres per day, with a normal range of specific gravities following water deprivation and loading. The reaction was persistently acid, and casual observations showed the pH to be between 5·6 and 6·4. Plasma electrolyte concentrations and her electrocardiogram were normal (Figures VII and VIII). X-ray examination of her chest showed a significant decrease in the heart size as judged by the cardio-thoracic ratio. Pulmonary congestion had disappeared. On this admission to hospital no further studies were made of plasma or urinary steroids. The changes in the relevant abnormalities before and after the removal of the adrenal adenoma are shown in Table I and Figure VII.

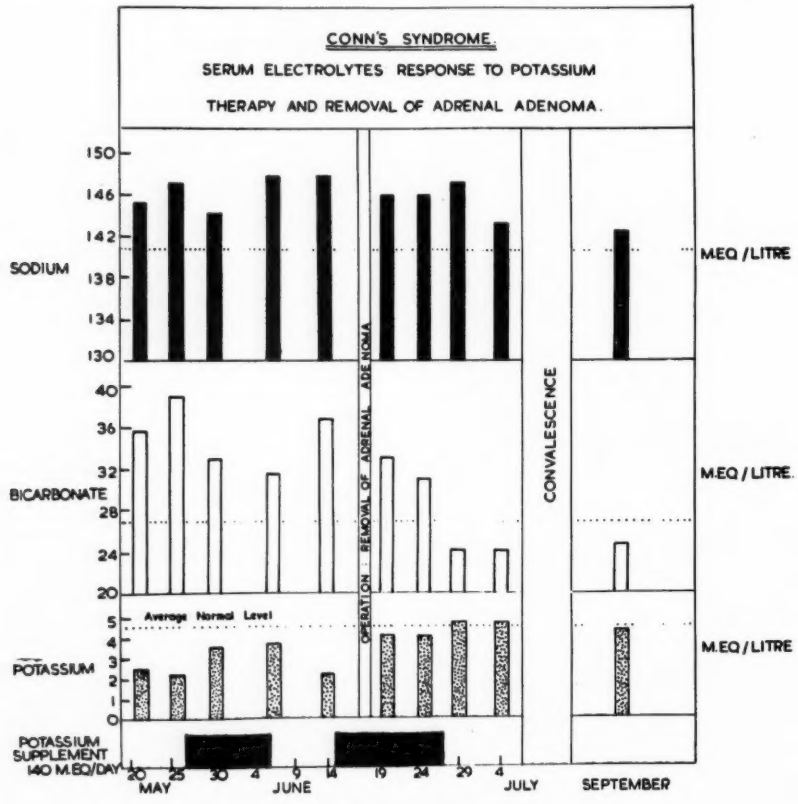

FIGURE VII

Serum sodium, potassium and bicarbonate values before, immediately after and three months after the removal of the adrenal adenoma. The response of these values to potassium supplement is also shown

THE DETERMINATION OF STEROIDS IN PLASMA AND URINE

The urinary 17-hydroxysteroids were determined by the method of Reddy *et alii* (1952) modified by applying the Porter-Silber colour reaction to the butanol extract of urine (Jenkins *et alii*, 1955). The 17-ketosteroids were determined by a method which is substantially that recommended by the Medical Research Council (1951).

Plasma 17-hydroxysteroids were determined in the following manner. Plasma, diluted with

one volume of water, was extracted three times with an equal volume of ethyl acetate. The combined extracts were washed with 0·1N sodium hydroxide and water. The volume of ethyl acetate was then reduced to one millilitre or less by evaporation under reduced pressure at 45° C. The steroids in this extract were concentrated by the chromatographic procedure of Chen *et alii* (1955). The extract was transferred to a paper strip, on which the fats and phospholipids were separated from the steroids by their differential movement in hexane and methanol. The fats moved to the top of the paper with the hexane front, leaving the steroids at the origin, from which methanol was used to move the steroids to a predetermined line part-way up the paper. The area around which the steroids were concentrated was then cut out and the steroids eluted from the paper with methanol. The concentration of steroids was determined by the Porter-Silber reaction using hydrocortisone as a standard, the estimations being performed with a Unicam spectrophotometer (SP 500) at a wavelength of 410 mμ. An additional check was made by using the reaction of fluorescence with sodium hydroxide, with hydrocortisone as the standard. The reaction was measured in a Unicam spectrophotometer with incident light at 475 mμ and reading the intensity of the emergent light at 557 mμ, using a photomultiplier unit for the measurement of this latter value.

For the determination of aldosterone in plasma, a total of 250 millilitres of plasma was extracted with ethyl acetate in the manner described. The steroids were concentrated by preliminary chromatography, using hexane and methanol. The steroid front was eluted with methanol, and the individual steroids were separated by the method of Bush (1952), using toluene/aqueous methanol and benzene/aqueous methanol as the chromatographic systems.

Two soda fluorescent spots were seen in each system, the first and larger one corresponding to compound F. The other, smaller spot ran close to the compound E marker in the toluene/methanol system and had an R_F value of 0·44 (compound E: 0·36). This material

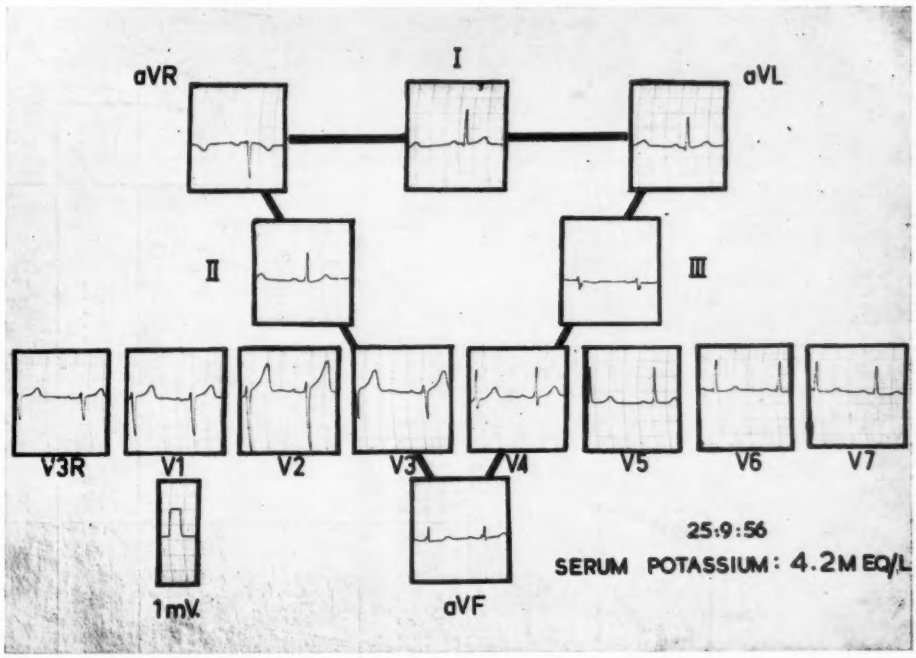

FIGURE VIII

The electrocardiogram three months after removal of the tumour. The serum potassium level was 4·2 milliequivalents per litre

was eluted from the paper, and its mineralocorticoid properties were demonstrated by its ability to reverse the urinary sodium/potassium excretion ratio in adrenalectomized mice, compound E with which it was most closely identified chromatographically being used as a control. Owing to the inavailability of radioactive sodium and potassium, no bioassay could be performed, but a quantitative determination was made by measurement of fluorescence, using compounds B, E and F as standards.

The determination of urinary aldosterone was made in a similar manner: urine was extracted three times with an equal volume of chloroform at pH 1. The extracts were washed with water and 0·1N sodium hydroxide, dried and subjected to paper chromatography, using the toluene/aqueous methanol system described by Bush (1952). A compound with chromatographic characteristics similar to those of the smaller spot found in the plasma was found in the urine. Its injection into adrenalectomized mice caused a similar reversal of the sodium/potassium excretion ratio.

TABLE I

M.C. A Summary of the Relevant Findings Before and After Removal of the Adrenal Adenoma

Investigation	Before Operation (May, 1956)	After Operation (September, 1956)
Blood pressure (millimetres of mercury)	185/95–240/140	120/80–145/100
Trousseau's sign	Positive	Negative
Serum potassium content (milli-equivalents per litre)	2·1	4·5
Serum sodium content (milli-equivalents per litre)	147	142
Serum bicarbonate content (milli-equivalents per litre)	39·0	25·0
Blood pH	7·55	7·41
Plasma steroids:		
17-Hydroxysteroids (micro-grammes per 100 millilitres)	9·6	10·2
Aldosterone (microgrammes per 100 millilitres)	2·1	Not detected (July, 1956)
Urine volume	3500–5500	1100–1650
Urine specific gravity	1005–1016	1024
Urine pH	6·95–7·6	5·1
Urinary steroids:		
17-Ketosteroids (milligrammes per twenty-four hours)	14·1	14·2
17 - Hydroxysteroids (milli-grammes per twenty-four hours)	9·4	3·4
Aldosterone (microgrammes per litre)	10·8	Not detected (July, 1956)

Cope and Garcia-Llaurado (1954) describe the finding of a potent mineralo-corticoid in the urine of a patient who was thought at that time to be suffering from potassium-losing nephritis (Evans and Milne, 1954). After submitting an extract of forty-eight-hours urine from this patient to paper partition chromatography, using a toluene/propylene-glycol system, they observed a spot which was located approximately at the RF value of compound E. This spot, when eluted from the chromatogram, was shown to possess potent sodium-retaining activity, whereas compound E was without such an effect when measured by its ability to reverse the Na^{24}/K^{42} ratio in the urine of adrenalectomized rats.

TABLE II

M.C. Showing the Changes in Response to Water Load and Pitressin Following the Removal of Adrenal Adenoma

Time	Procedure	Urinary Findings: Volume (Millilitres)	Urinary Findings: Specific Gravity
10/6/56:			
8.00	Fourteen hours' fluid deprivation	—	1017
8.05	Water load, one and a half litres.		
9.00		420	1002
9.30		580	1000
10.00		290	1002
11.00		300	1003
12.00		190	1008
16/6/56:			
8.00	Fourteen hours' fluid deprivation	—	1016
8.05	Water load, one and a half litres, 10 international units of Pitressin.		
9.00		340	1004
9.30		470	—
10.00		310	1006
11.00		320	1008
12.00		240	1008
8/7/56:			
8.00	Fourteen hours' fluid deprivation	—	1018
8.05	Water load, one and a half litres, 10 international units of Pitressin.		
9.00		20	—
10.00		70	1016
11.00		60	1017
12.00		50	1022

DISCUSSION

The clinical and biochemical features of this patient are similar in most respects to those of the patient originally reported by Conn (1955). The syndrome was manifest in its fully developed state by the presence of hypokalæmia, mild hypernatræmia, alkalosis, hypertension and a defect in the tubular reabsorption of water. Excessive amounts of mineralo-corticoid were found in the urine, in the plasma and in the tumour removed at operation. The evidence presented suggests that this was aldosterone, but its complete and final identification was not possible.

A significant finding in this syndrome is that of hypokalæmia and alkalosis. Lowering

of plasma potassium results almost certainly from the direct effect of aldosterone on the renal tubules. Studies with aldosterone have shown that it possesses marked sodium-retaining and potassium-depleting properties (Prunty *et alii*, 1954; Kekwick and Pawan, 1954; Hetzel *et alii*, 1956). In our patient, a potassium load was accompanied by a prompt increase in the renal excretion of this ion, so that on two occasions as much as 80% of the additional administered potassium was lost in the urine cells, with displacement of sodium to its normal extracellular site and subsequent excretion by the kidney.

The reason for the development of alkalosis in such patients is not clear. The studies of Cooke *et alii* (1952) indicate that the alkalosis is secondary to the lowered serum potassium content. They have postulated that, associated with potassium depletion, there is a movement of sodium and hydrogen ions from the extracellular to the intracellular fluid in the

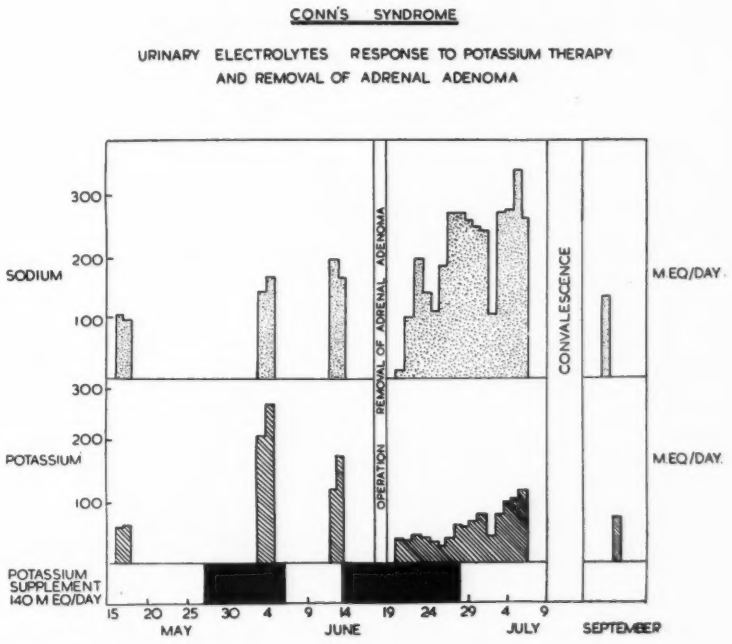

FIGURE IX

The changes in urinary electrolytes following the exhibition of potassium supplement before and after removal of the adrenal adenoma

(Figure IX). Evidence concerning the extent of the decrease in the potassium tolerance in this patient is lacking in the absence of more detailed balance studies. The fall in the urinary potassium excretion following the removal of the tumour testifies to the influence of this hormone on the renal excretion of potassium.

The prompt diuresis of sodium following the removal of the tumour is also an indication of the profound effect of aldosterone on the excretion of this element. It is of interest that when additional potassium was administered pre-operatively, there was an increase in the renal excretion of sodium. This may indicate some movement of potassium into approximate ratio of 2 : 1, the overall movement in hydrogen ions from the extracellular fluid resulting in alkalosis.

An attempt was made to determine whether this was the sequence of events in this patient. She was given an alkaline potassium salt (potassium citrate) to determine whether, with an increase in plasma potassium content and, presumably, in intracellular potassium, the alkalosis could be corrected. Figure VII shows that on this régime, with an elevation of plasma potassium content, there is a lessening of the alkalosis. There has also been an increase in the sodium concentration of the extracellular fluid, due, possibly, to a movement of this ion

from the cells. Although we have no studies on the ionic concentration of intracellular components to support this contention, the evidence presented is suggestive but by no means conclusive.

Thirst and polyuria were conspicuous complaints in this patient's history, in which polyuria preceded her thirst. The precise mechanisms for these symptoms have not been fully elucidated. There is no good evidence to support the contention that the excretion of large volumes of urine had an osmotic basis. Throughout the whole period of study, we were impressed with the fact that the urine was dilute, as indicated by its consistently low specific gravity. The history was similar to that of mild *diabetes insipidus*, and her failure to respond to 10 units of pitressin preoperatively suggests an inability of the renal tubule to respond to this hormone in the presence of excessive aldosterone secretion. Her response to pitressin following the removal of the tumour was normal, in that the diuresis following water loading was inhibited by the administration of 10 units of pitressin.

Conn, in his original paper (Conn, 1955), makes brief reference to the fact that pitressin did not correct this failure of renal concentrating ability. Whether pitressin fails because of structural changes in the renal tubule associated with chronic hypokalæmia, or because of direct antagonism between the two hormones, is not known. In this patient the urine volumes decreased quite soon after operation, so that on the fifth post-operative day the volume had fallen to 1570 millilitres and the specific gravity had risen to 1016. This would appear to be too sudden for the correction of a structural abnormality and suggests that either the tubule was responding abnormally as a result of some functional defect, or perhaps that, with a normally responsive tubule, the actions of aldosterone and antidiuretic hormone in respect to water excretion are antagonistic.

Since primary aldosteronism is in most instances a curable condition, its recognition is extremely important, and the clinical diagnosis merits discussion. Early diagnosis is stressed, not only because of the desirability of relief of symptoms and the prevention of damage to tissues from hypertension, but also because the underlying disease may be a tumour which may subsequently show malignant behaviour, as in the case described by Foye and Feichtmeir (1955). The symptoms—those of mild hypertension, polyuria and weakness—are not specific, and the clue to the diagnosis may be given only by the striking biochemical findings, particularly the low serum potassium content.

Prior to the recognition of primary aldosteronism as a clinical entity, a number of patients had been described with lowered serum potassium levels which had been ascribed to renal disease (Earle *et alii*, 1951; Evans and Milne, 1954; Wyngaarden *et alii*, 1954). It is of interest to consider whether these may not have really been instances of primary aldosteronism. In the patients described by Evans and Milne (1954) and by Wyngaarden *et alii* (1954), hypertension and alkalosis were observed in addition to hypokalæmia. In the former patient, Cope and Garcia-Llaurado (1954) found excessive excretion of mineralo-corticoid in the urine, and the patient was subsequently found to have an adrenal adenoma. This patient was in fact suffering from what is now recognized as Conn's syndrome. In the patient described by Wyngaarden *et alii*, post-mortem examination showed the necrotizing arteriolitis of malignant hypertension, but there was no evidence of primary renal disease. The adrenals, the combined weight of which was 20·5 grammes, were said to be normal. In our experience these are large adrenals for a sick person whose body weight was less than 120 pounds. Although they were reported to be histologically normal, it is possible, in the light of recent knowledge, that this may have been an example of excessive activity of the *zona glomerulosa*. This could result in hypokalæmia, hypertension and secondary renal damage, as in the patient reported by Van Buchem *et alii* (1956). Differentiation from primary renal disease with secondary hypokalæmia would be difficult or impossible without steroid studies such as were employed in our patient.

In other patients, it seems likely that the hypokalæmia was secondary to renal disease. In the patient described by Earle *et alii* (1951), although the final diagnosis is unknown, persistent albuminuria and acidosis indicate primary renal disease. One of us (B.H.) has seen two patients with severe muscular weakness and low serum potassium content; and in both instances marked impairment of renal function was evident as shown by nitrogen retention, acidosis and hyperchloræmia. Both patients manifested osteomalacia. Although no special steroid studies were made in these patients, the presumptive diagnosis of a primary renal lesion seems justified. The presence of underlying renal disease may not always be as apparent in these cases. Mahler and Stanbury (1956) reported a case of potassium-losing renal disease in which the blood urea level was

normal, proteinuria was minimal and occasionally absent on routine testing, and, despite an inability to produce maximally acid urine, there was no metabolic acidosis. The biochemical abnormalities were corrected, and all the symptoms but nocturia were relieved by administration of potassium bicarbonate and a diet of relatively low sodium content. The primary renal disease was shown by biopsy to be chronic pyelonephritis. In such cases, steroid studies as performed in our patient would be a valuable aid to diagnosis.

Hypokalæmia may occur in other conditions, and this may lead to a suspicion of excessive secretion of aldosterone. One of these is familial periodic paralysis. The family history, early age of onset, complete return of muscle strength and serum potassium content to normal between attacks, absence of alkalosis and absence of any apparent renal defect all serve to differentiate between the two conditions. In doubtful cases we believe that the labour of steroid determinations is warranted for their positive differentiation.

Schwartz and Relman (1953) have drawn attention to the occurrence of hypokalæmia, with associated symptoms, resulting from chronic diarrhœa induced by the excessive use of laxatives. In the two patients they reported, in one of whom alkalosis was present, an abnormality of renal excretion was present. This was manifest as an impaired ability to concentrate urine, and depression of glomerular and tubular function as revealed by clearance studies. In contrast to our patient, the urinary excretion of potassium was very low (three to nine milliequivalents per day). We are aware of a similar patient with chronic hypokalæmia in whom chronic diarrhœa is the probable diagnosis; this was strongly suggested by the persistently low urinary potassium excretion (Whyte, 1956).

In its fully developed form the symptoms of primary aldosteronism—hypertension, polyuria, attacks of weakness—are well marked. Now that the existence of this condition is appreciated, the diagnosis may be suspected on clinical grounds; this leads to search for further evidence from alterations in serum electrolytes, particularly in the serum potassium. Proof of the diagnosis will depend on the demonstration of increased production of aldosterone. However, this diagnosis may be difficult in the early stages of the patient's illness. As in our patient, the symptoms and signs may be those of essential hypertension, and even the electrocardiogram may not show any characteristic features. As we have already stressed, it is desirable that the diagnosis be made as early as possible. The condition should therefore be suspected in patients with unexplained (or "essential") hypertension, and we would suggest that an examination of serum electrolytes be included in the routine investigational procedure in these patients, particularly if the hypertension cannot be readily controlled by antihypertensive drugs, if the symptoms are unrelieved, or if new symptoms appear.

In conclusion, there is no doubt that the syndrome originally described by Conn is well authenticated. The clinical and biochemical features of the patient which we have described could well be called classical, since they closely resemble those present in Conn's original patient. It is apparent that not all patients with primary aldosteronism present such a simple problem with respect to diagnosis or treatment. As in Cushing's syndrome, so in Conn's syndrome the pathological lesion may be a benign or malignant tumour or bilateral hyperplasia. It has been suggested that the case reported by Wyngaarden *et alii* (1954) as a case of renal disease was due to excess aldosterone production resulting from bilateral adrenal hyperplasia; the seventeen-year-old male patient in the case reported by Van Buchem *et alii* (1956) is stated to have had bilateral adrenal enlargement; the patient in the case reported by Foye and Feichtmeir (1955) died as a result of malignant disease. It is important, therefore, to make the diagnosis, not only for the relief of hypertension or hypokalæmia, but also with the realization that the underlying adrenal lesion may be a malignant rather than a benign tumour.

ACKNOWLEDGEMENTS

We are grateful to Professor M. R. Ewing for his advice and help with the management of this patient. Dr. Donald Emslie-Smith first suggested the diagnosis from inspection of the electrocardiogram. Miss June Sheath, B.Sc., performed many and varied biochemical procedures. We should also like to thank the ward sisters and staff for their cooperation and help.

Part of the expenses for the steroid investigations in this study were defrayed by a generous grant from the Upjohn Company, Kalamazoo, Michigan, who also supplied reference steroids used in chromatographic procedures.

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Proceedings of The Royal Australasian College of Physicians

ANNUAL MEETING, 1957

The Annual Meeting of the College in 1957 was held in Brisbane, Queensland, from May 29 to 31. It was attended by Fellows and Members from throughout the Commonwealth and the Dominion. The President, Dr. E. G. Sayers, was in the chair.

College Ceremony

The Annual Ceremony of the College was held in the Great Hall of the Brisbane Grammar School on Wednesday, May 29, 1957, in the presence of His Excellency the Administrator of the Government of Queensland, the Honourable Mr. Justice Mansfield. An audience of 400 was present.

Addresses were given by the President and His Excellency the Administrator, after which newly admitted Fellows and Members were presented to the President. Professor H. C. Webster, Ph.D. (Cantab.), D.Sc. (Tas.), F.Inst.P., Dean of the Faculty of Science in the University of Queensland and Convener of the Australian National Committee for the International Geophysical Year, then delivered an address entitled "The Upper Atmosphere". Guests were entertained at supper after the ceremony.

Scientific Sessions

Three scientific sessions were held in the Main Lecture Theatre of the Medical School, Herston. One session, the Harveian Commemorative Session, honoured the tercentenary of the death of William Harvey. At this session the following contributions were given: "William Harvey", by Professor E. Ford; "Clinical Problems of Limb Circulatory Stress", by J. R. S. Lahz, F.R.A.C.S.; "*De Motu Cordis*: Cellular Physics and Chemistry", by Professor W. V. Macfarlane; "On the Nature of Dyspnœa in Mitral Stenosis", by B. C. Sinclair-Smith; "An Interpretation of the Left Cardiac Border aided by Angiocardiographic Studies", by E. J. Halliday. An exhibition of books was also arranged. These included a copy of Harvey's *Opera Omnia*, presented to the College by the Royal College of Physicians of London for the occasion, and of the *Exercitatio Anatomica* and a facsimile of Harvey's Padua diploma, forwarded by the London College for the exhibition.

The following contributions were given at other scientific sessions: "Atypical Congenital Hæmolytic Anæmia", by G. C. de Gruchy; "The Adrenocortical Response to Intravenously Administered ACTH in Hypocorticism and Suspected Instances of Hypocorticism", by A. W. Steinbeck; "Neurological Manifestations of Hypoparathyroidism and Their Pathogenesis—Further Experimental Studies", by Lyal Watson; "Studies on the Active Transport of Glycine by Small Intestine", by W. J. Hensly; "Reserpine and Extracellular Fluid Volume", by R. A. Melick and Maurice McGregor (presented by R. A. Melick); "The Diffusing Capacity of the Lung in Health and Disease", by R. B. Blacket and R. A. B. Holland (presented by R. A. B. Holland); "The Experimental Production of the Hamman-Rich Syndrome", by John Read; "Toxoplasmosis in Queensland", by E. H. Derrick; "The Nature of Hormone Induced Eosinopenia", by Bryan Hudson (see page 228).

The following summaries have been received from individual contributors.

E. H. Derrick, in a paper on "Toxoplasmosis in Queensland", stated that the protozoon, *Toxoplasma gondii*, could infect a wide range of animals and birds, as well as man. Infection typically passed through two stages. There was first a parasitæmic stage, and then, if the host survived, a chronic or latent stage, in which pseudocysts might persist in brain or heart or other organ. In mice the parasitæmic stage might last about five months. Human infection might be congenital or acquired. Congenital toxoplasmosis, which might occur if pregnancy coincided with the parasitæmic stage, was estimated to occur in England in at least one in 35,000 births. Acquired toxoplasmic infection was common as shown by serological tests, but was usually symptomless. However, fatal cases had occurred. The commonest clinical manifestation was lymphadenopathy, with or without fever. It was suggested that pneumonitis might occasionally be of toxoplasmic origin. In the latent stage, rupture of a pseudocyst could cause an intense focal inflammatory reaction. In the discussion, J. H. Pope reported that *Toxoplasma* had been isolated from 40% of bandicoots caught in and near Brisbane and in North Queensland, and also from *Rattus assimilis* and *Rattus norvegicus*. In transmission experiments, pseudocysts had been found highly infective for mice *per os*. I. Cook had carried out dye and complement-fixation tests. The latter test, which was technically simpler, had proved reliable with bandicoot sera when checked against isolation results. In a series of normal human sera in Queensland, 16% had contained complement-fixing antibodies.

R. A. Melick and Maurice McGregor, in a paper presented by R. A. Melick, discusses, "Reserpine and Extracellular Fluid Volume". It was noted that a gain in weight during the taking of reserpine had often been observed. Several clinical reports had described

fluid retention with reserpine ; those had suggested that the weight gain in patients on reserpine might have been partly due to fluid retention. Serum sodium content, hæmatocrit value and extracellular fluid volume using Br^{82} had been measured in 18 hypertensive patients before and at the end of a course of treatment with reserpine. Most patients had gained weight whilst taking reserpine, but the extracellular fluid volume, expressed as a percentage of body weight, had not changed. Serum sodium content had been unaltered, but hæmatocrit values had fallen in most patients, and a significant negative correlation had been found between changes in hæmatocrit value and changes in extracellular fluid volume. It was concluded that, in these patients, no fluid retention had occurred with reserpine, but that fluid retention in some cases could not be excluded. The cause of the fall in hæmatocrit values was uncertain, but was possibly due to a rise in plasma volume.

John Read, presented a paper on " The Experimental Production of the Hamman-Rich Syndrome ", in which it was reported that rabbit anti-rat-lung serum had been prepared by immunization of rabbits with suspensions of blood-free rat lung. Intrabronchial injection of such serum had led to a distinctive series of pulmonary changes in rats, designated as " pneumonotoxic pneumonia ". An initially exudative and proliferative process involving alveolar septa and spaces had progressed to a phase of alveolar wall fibrosis, with intra-alveolar organization distinctly secondary in degree and extent. Hyperplasia of alveolar lining cells, peculiar granulomatous buds on the alveolar septa, and rare foci of tissue necrosis had also been seen. Morphologically the appearances had been identical with those seen in human cases of the Hamman-Rich syndrome at all stages at which they could have been compared.

Lyal Watson, in a paper on " Neurological Manifestations of Hypoparathyroidism and their Pathogenesis—Further Experimental Studies ", reviewed in brief the neurological features of uncontrolled hypoparathyroidism. He said that manifestations which resulted from involvement of the central nervous system included mental changes, epileptic fits, raised intracranial tension and papilloedema, coma, electroencephalographic abnormalities and intracranial calcification. Some of the changes, including epileptic fits and raised intracranial tension, had been produced experimentally in rabbits by prolonged hypocalcæmia. The techniques and results of the studies were described and discussed in some detail. It was concluded that the central neurological phenomena of hypoparathyroidism were due to hypocalcæmia. There were at least two different mechanisms involved in their pathogenesis, and these might occur together or separately. Fits were associated with mild cerebral œdema. Raised intracranial tension was not due to cerebral œdema and had an osmotic basis, resulting from a disorder of the normal active secretion of cerebrospinal fluid.

CLINICAL MEETING

A clinical meeting was held at the Main Lecture Theatre, Medical School, Herston.

COLLEGE DINNER

The College Dinner was held at Rowe's Cafe, Brisbane, on the evening of Thursday, May 30, 1957. The toast of the College was proposed by the Chairman of the Queensland State Committee, Dr. Ellis Murphy, and acknowledged by the President. Dr. C. R. Burns, the Vice-President for New Zealand, proposed the toast of the guests representing sister Colleges. Mr. K. B. Fraser, F.R.A.C.S., replied.

OFFICE-BEARERS

The following is the constitution of the Council for the year 1957-1958 :

President : E. G. Sayers.

Vice-Presidents : Bruce Hunt, Clive Fitts and C. R. Burns (New Zealand).

Censor-in-Chief : T. M. Greenaway.

Honorary Treasurer : W. P. MacCallum.

Honorary Secretary : H. Maynard Rennie.

Immediate Past President : C. G. McDonald.

Elected Councillors : Fellows : Sir Charles Blackburn, M. E. Chinner, J. E. Clarke, Professor Lorimer Dods, Clive Fitts, T. M. Greenaway, Professor J. G. Hayden, Bruce Hunt, Guy Lendon, Sir Alexander Murphy, K. B. Noad and Morvyn Williams ; Members : Bryan Hudson and D. S. Stuckey.

Executive Committee, 1957-1958 : E. G. Sayers (President), C. H. Fitts (Chairman), W. P. MacCallum (Honorary Treasurer), H. Maynard Rennie (Honorary Secretary), T. M. Greenaway, C. G. McDonald, Sir Alexander Murphy and K. B. Noad.

Assistant Honorary Secretary, 1957-1958 : G. L. McDonald.

Board of Censors

Censor-in-Chief : T. M. Greenaway.

Australian Board : J. M. Bonnin, J. E. Clarke, J. L. Frew, W. E. King, K. B. Noad and A. W. Morrow.

New Zealand Board : Professor F. H. Smirk (Senior Censor for the Dominion), C. R. Burns, Professor J. E. Caughey, J. F. Landreth, E. H. Roche and J. M. Twhigg.

Committees

Editorial Committee of " Australasian Annals of Medicine " : Dr. C. G. McDonald was appointed Chairman of the Editorial Committee for 1957-1958, and Dr. H. M. Whyte was elected a member of the Committee for this period.

Research Advisory Committee : The following were appointed to the Research Advisory Committee for 1957-1959 : Professor Lorimer Dods (Chairman), Dr. S. A. Smith, Professor C. R. B. Blackburn, Sir Macfarlane Burnet, Professor J. C. Eccles, Professor E. Ford, Dr. D. S. Stuckey (Honorary Secretary). Ex-officio members : The President, the Honorary Secretary and the Honorary Treasurer.

MEMBERSHIP

Admission of Fellows. The following Fellows were admitted on May 29, 1957, after election by the General Body of Fellows: *under Article 44:* R. R. H. Lovell, of Victoria; *under Article 42:* H. W. Johnson, W. G. Livingstone, J. H. Tyrer, L. D. Walters and H. G. Wilson, of Queensland; G. V. Hall, A. J. May and T. E. H. Spark, of New South Wales; G. C. de Gruchy and H. J. Sinn, of Victoria; R. C. Angove, W. M. Irwin and R. A. A. Pellew, of South Australia; J. L. Adams and J. D. Willis, of New Zealand.

Admission of Members. The following candidates who were successful at examinations held in New Zealand in February, 1957, and in Brisbane and in May, 1957, were admitted to Membership on May 28, 1957: D. J. Deller, A. H. Gibson, D. J. Harbison, E. J. Lines and A. P. Skyring, of New South Wales; P. D. Breidahl, B. M. King, I. H. McKenzie, M. L. Mashford and P. J. Nestel, of Victoria; H. A. Copeman, N. J. Nicolaides and W. S. Rowe, of Queensland; N. H. Brooke, D. G. Campbell, D. R. Hay, I. C. Isdale, W. B. Jackson, B. J. Kelly, G. B. Kiddle, R. G. Lawrence, C. M. Luke, C. A. MacLeod, J. D. K. North, L. H. Stevens and R. C. Tait, of New Zealand. R. A. Barter of Victoria was admitted to Membership under the provisions of Article 37.

Honour. The honour of Commander of the Order of the British Empire was bestowed by Her Majesty the Queen upon Dr. Ralph Whishaw, of Hobart.

Obituary. The Council records with regret the death of Dr. A. W. Holmes à Court, of Sydney, a Foundation Fellow of the College and President for 1950-1952; Dr. George F. Strong, of Vancouver, an Honorary Fellow of the College; Sir Lionel Whitby, of Cambridge, who had visited Australia and New Zealand in 1956 as Sims Commonwealth Travelling Professor; Dr. D. W. Carmalt-Jones, of England, a Foundation Fellow and the first Dominion Vice-President; Dr. G. R. Kirk, of Christchurch, and Dr. Otto S. Hirschfeld, of Queensland, who were Fellows of the College; and Dr. Stewart Hunter, of Dunedin, Dr. G. A. W. Johnston, of Sydney and Dr. W. J. Freeman, of Hobart, who were Members.

Membership Roll. The College now has a roll of 326 Fellows and 494 Members.

GENERAL

Representatives of the College. The following have been appointed as College representatives: Sir Alexander Murphy and Professor J. G. Hayden to participate in the Colloquia on Hypertension and Pregnancy Toxæmia; Professor J. E. Caughey as Examiner in Medicine in New Zealand for the Fellowship of the Faculty of Radiologists; Professor F. H. Smirk on the New Zealand Medical Research Council (a reappointment).

Future Meetings of the College. The venue of future meetings of the College would be as follows: 1957—Ordinary Meeting, Sydney; 1958—Annual Meeting, Sydney; 1959—Annual Meeting, Adelaide, and Ordinary Meeting, Melbourne; 1960—Annual Meeting, Melbourne, and Ordinary Meeting, Perth.

Therapeutics Assessment Committee. Council had received requests from certain manufacturing chemists that the College undertake the clinical trial of new remedies, particularly those which were produced in Australia. After careful investigation by a committee it had been decided to set up a Therapeutics Assessment Committee to undertake the clinical trial of such remedies, under certain specified conditions.

Sir Arthur Sims Commonwealth Travelling Professors. Sir James Paterson Ross, Professor of Surgery in the University of London, visited Australia and New Zealand as Sir Arthur Sims Commonwealth Travelling Professor from March to May, 1957, his itinerary being arranged by the Royal Australasian College of Surgeons. The appointment has been announced of two Sims Travelling Professors for 1958. They are Professor M. L. Rosenheim, C.B.E., M.D., F.R.C.P., of London, who will visit Australia and New Zealand, and Professor R. M. Janes, F.R.C.S. (*C*), F.A.C.S., Hon. F.R.C.S., of Toronto, who will visit parts of Africa.

Overseas Tour by President. The President, Dr. E. G. Sayers, was the guest of the American College of Physicians at its Annual Meeting in Boston in April, 1957, and was honoured to receive an Honorary Fellowship of the College. The President informed the Council of this College upon his return of the hospitality shown him and the many courtesies which he received during his visit to the United States of America. The President presented to the American College on behalf of this College a gavel carved from New Zealand wood two thousand years old, and this gift was received with appreciation by the American College. Upon the completion of his visit to the United States the President visited the United Kingdom and was further graciously entertained by the Royal College of Physicians of London and the Royal College of Physicians of Edinburgh. He returned to attend the Annual Meeting in Brisbane on May 27. Council recorded its appreciation of the courtesies shown to the President of the College throughout his tour.

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SEAMER AND ARUNDEL STS., GLEBE, SYDNEY